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ASSESSMENT OF FUTURE ENVIRONMENTAL TRENDS AND PROBLEMS: AGRICULTURAL USE OF APPLIED GENETICS AND BIOTECHNOLOGIES

by

George H. Kidd, Melanie E. Davis, and
Parviz Esmailzadeh
Battelle Columbus Laboratories
505 King Avenue
Columbus, Ohio 43201

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Contact No. 68-02-3640

Project Officer

Morris Levin
Office of Stategic Assessment and Special Studies
Environmental Protection Agency
Washington D.C. 20460

OFFICE OF STRATEGIC ASSESSMENT AND SPECIAL STUDIES
OFFICE OF RESEARCH AND DEVELOPMENT
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16. ABSTRACT

Battelle's Columbus Laboratories will identify and define future environmental concerns arising from applying genetic engineering technology to agricultural problems. Two genetic engineering technologies, plant tissue culture and recombinant DNA, will be considered. Potential environmental problems resulting from the research process (e.g., release or escape of recombinant organisms into the environment, dispersion or reproduction of recombinant organisms or molecules) and from applications of genetically engineered organisms (e.g., large-scale cultivation of a nitrogen-fixing grain crop) will be assessed. Information pertaining to potential problems will be obtained from the published scientific literature and from telephone interviews of experts currently active in applied genetics research. A cross-impact analysis program/model will be used to identify and define some impacts and trends, e.g., such as political, economic, and social settings which facilitate the agricultural applications of recombinant DNA. In addition to identification and definition of environmental impacts, other outputs would include identification of organizations and individuals interested or involved in agricultural genetic engineering, descriptions of trends that may affect public health or environmental policy, and identification of major data gaps and inadequacies in analytical methods.

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ABSTRACT

This research program was initiated with the overall objective of providing information on trends and potential future environmental problems related to agricultural genetic engineering. The information is for use by those engaged in planning EPA's environmental research programs and by other interested individuals.

The report is organized into three sections. Section 1 states the problem and describes the current regulatory background.

Section 2 defines the scope of the problem by discussing the vectors and genes which potentially could be used to modify the genetics of agronomic plants. Vectors discussed are Agrobacterium (Ti plasmids) and plant viruses. Gene clusters discussed are nitrogen fixation (nif), hydrogen uptake (hup), water-splitting (lit), denitrification (den), osmoregulatory (osm), and carboxylase (cfx) genes. Mutation induction using plant tissue culture techniques is also discussed. For each, state-of-the-art research for using recombinant DNA techniques and potential environmental benefits and hazards of applying these techniques are discussed.

The biochemistry of nitrogen fixation and the location of the nif gene cluster in K. pneumoniae are relatively well understood. Large plasmids that encode the ability to metabolize hydrogen in several species of hydrogen bacteria have been identified, and these have been transferred among microorganisms. Plasmids that encode salt tolerance have also been identified and transferred among microorganisms. Locations of the genes controlling the processes, such as denitrification and carbon dioxide fixation, are under investigation. Much basic research is still needed to understand the physiology/biochemistry of these processes. Potential environmental benefits of agricultural genetic engineering are many while hazards are few.

This report briefly identifies gaps or inadequacies in the present knowledge of the problem and research or method development required to fill them. These are presented as potential future research areas for consideration by those engaged in planning EPA's environmental research program.

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SECTION 1

PROBLEM IDENTIFICATION AND REGULATORY BACKGROUND

PROBLEM IDENTIFICATION

This report provides information on trends and potential future environmental problems and opportunities related to agricultural genetic engineering. This information is for people engaged in planning EPA's environmental research programs and for other interested individuals. The report is concerned primarily with recombinant DNA technology. Tissue culture technology is mentioned briefly, and conventional crop breeding is not included.

By most accounts, a prominent aspect of the technological trajectory of the 1980s will come from continued advances in molecular biology and genetic engineering. Applications of these technologies will cause profound social, economic, environmental, ethical, legal, and cultural impacts. It is imperative, therefore, that the poorly understood and potentially massive ramifications of the new technology be anticipated before they are put to use.

Current research on agricultural applications of recombinant DNA is directed toward augmenting or establishing several physiological capabilities in crop plants. These capabilities include nitrogen fixation, enhanced photosynthesis, and adaptation to environmental stresses. These research efforts must lead to greater crop productivity because, for example, it is estimated that developing countries will experience a 145 million ton deficit in staple food crops (approximately 20 percent of the total food demand in these countries) by 1990 (Mellor, 1979). Improved worldwide crop performance has become particularly important for several other reasons as well: the worldwide exponential population growth; the continued loss of agricultural land to urbanization, erosion and desertification; the increasing scarcity of energy for pesticides, fertilizers, irrigation, and mechanization; and the continued disruption of ecosystems by industrial waste products.

ORGANIZATION OF REPORT

This report consists of three sections. Section 1 states the problem: namely, that the ramifications of genetic engineering must be anticipated so that appropriate research can be conducted and regulatory measures taken. Also, in this section the current regulatory background for agricultural genetic engineering is described.

Section 2 defines the scope of the problem by discussing each vector and gene cluster which potentially could be used to modify the genetics of agronomic plants. The state-of-the-art for using recombinant DNA techniques as well as potential environmental benefits and hazards of using these techniques are discussed for each vector and gene. Using this information obtained from the literature and information gathered from interviews with experts in plant genetic engineering, cross-impact analysis was performed, and a scenario constructed that forecasts and describes the interactions between events and predictions of the likelihoods and extents to which these events will occur. The meager historical data base makes risky any attempt to project the future. The emphasis, therefore, has been to identify the sources and general magnitude of trends, rather than attempt precise numerical estimates.

Finally, Section 3 identifies gaps or inadequacies in the present knowledge of the problem and the research or method development required to fill them. These are presented as potential future research areas for consideration by those engaged in planning EPA's environmental research program.

There are four appendices to this report: a partial list of scientific conferences on plant genetic engineering (Appendix A); a partial list of people involved in, interested in, or impacted by agricultural genetic engineering (Appendix B); a vertifile of abstracts of recent popular articles on plant genetic engineering (Appendix C); and a description of methodology used to generate the scenario on the impact of genetic engineering on agriculture in 1990 (Appendix D).

DEFINITION OF GENETIC ENGINEERING

Recombinant DNA, a technology that is part of genetic engineering, is an approach to controlling hereditary traits by manipulating the genetic material of organisms. Some terms associated with recombinant DNA technology are defined in Table 1.

Recombinant organisms may acquire capabilities they do not normally possess. They may be able to adapt to or cope with an altered environment. Thus, nature's usual slow process of creating genetic variability may be directed and accelerated in the laboratory. Theoretically, recombinant DNA technology can be an excellent tool for changing the genetic complement of any organism -- plant, animal, or microbe. However, due to their greater genetic simplicity, more rapid growth, and the large data base on bacterial genetics and processes, recombinant DNA technology has thus far been most successfully applied to bacteria. There are three prerequisites to engineering any organism:

- A gene that can be identified, isolated, characterized, and cloned
- A vector to put the gene into a bacterium
- A host that will accept the gene and that will express the new gene to produce a protein.

TABLE 1. TERMS USED IN RECOMBINANT DNA TECHNOLOGY

Callus - tissue growing from the disorganized proliferation of cells excised from a plant.

Cloning - obtaining a group of genetically identical cells from a single cell. This term has assumed a more general meaning to include making many copies of a gene.

Differentiation - the process by which single cells grow into particular forms of specialized tissue, i.e., root, stem, leaf.

DNA - Deoxyribonucleic acid, the genetic material of all cells. It bears, encoded in its genes, the instructions for passing on hereditary characteristics.

Encode - to transmit the sequence of genetic information contained in the DNA.

Gene - pieces or fragments of DNA, each encoding particular characteristics of an organism.

Gene library - a collection of DNA fragments from organisms. So far, no simple way has been devised for sorting or cataloging the contents of gene libraries. However, DNA pieces can be moved into bacterial cells where sorting according to gene function becomes feasible.

Host - the organism, typically a bacterium, into which a gene from another organism is transplanted.

In Vitro - a Latin term meaning "in glass". Here, this term refers to the test-tube culture of cells derived from multicellular organisms.

Mutate - to bring about a change in the genetic constitution of a cell by altering its DNA. In turn, mutagenesis is any process by which cells are mutated.

Plasmid - a small, self-duplicating, circular piece of DNA that can be readily moved into bacterial cells. A desired gene can be inserted into a plasmid and the plasmid, in turn, can become a vehicle for moving the desired gene into a bacterium.

Recombinant bacteria - a type of microorganism whose genetic makeup has been altered by deliberate introduction of new genetic elements. The offspring of these altered bacteria also contain these new genetic elements.

Regeneration - manipulation of individual cells or masses of cells to grow into whole plants.

TABLE 1. (Continued)

Restriction enzymes - enzymes that recognize certain specific regions of a long DNA molecule and then cut the DNA into smaller pieces.

RNA - ribonucleic acid is a long molecule that is a copy of, but chemically different from, DNA. It constitutes an intermediate in the synthesis of protein.

Shotgun - the process of breaking up the DNA derived from an organism--a plant or animal, for example--and then moving each separate and unidentified DNA piece into a bacterium.

Suspension culture - individual cells or small clumps of cells growing in a liquid nutrient medium.

Transformation - the process of placing new genes into a host cell, thereby inducing the host cell to exhibit functions encoded by that DNA.

Vector - an object that is used to transport genes into a host cell. Vectors can be plasmids, viruses, or even other bacteria. A gene is placed in the vector, and then the vector "infects" the bacterium.

The process of producing recombinant organisms--E. coli is a commonly used host--is shown in Figure 1. It involves cloning, the process of placing a foreign gene into a cell so that the gene and its protein product are reproduced manyfold. Several steps are involved. First, DNA encoding for the desired new protein is identified in another organism or is synthesized. After this DNA is identified and isolated, it is cut into pieces. This is accomplished by utilizing restriction enzymes, which cut the long DNA molecule at specific points, thereby generating short DNA fragments. Breaking up the total DNA from a single organism into thousands of fragments is known as a shotgun treatment.

Next, the desired piece of DNA is inserted into the host cell via a vector--usually a plasmid. To enable them to act as vectors, the circular plasmids are opened, and the foreign DNA is inserted into the opening. The host is treated to encourage it to take up the recombined plasmids, which then multiply within the cell. The recombinant organism is transformed so that it can now use its new genetic information.

In short, the steps necessary to engineer a recombinant organism are:

- Identify and isolate DNA containing a gene of interest.
- Cut DNA containing the gene into pieces.
- Locate a vector and put gene in the vector.
- Infect an organism with the vector containing the gene.
- Encourage the host cell to multiply and express the gene.

These steps must occur in order, and each step must be completed before the next is begun.

Once engineered, recombinant cells can be, and are saved. Collections of millions of recombinant bacteria, each bacterium containing a different DNA fragment from another organism, have been made in several laboratories both in the U.S., Japan, and Europe. These collections are called gene libraries.

Plant tissue culture is another form of genetic engineering. Tissue cultures can take several forms including callus, suspension, and single cell cultures; but all cultures are produced by the same basic method. Cells, tissue, or organs are removed from a plant and grown in an artificially controlled environment on a growth medium containing precise quantities of mineral salts, sugar, vitamins, and a combination of plant hormones. This control allows the experimenter to manipulate the plant material to achieve the desired end result.

Perhaps the most remarkable aspect of plant tissue culture is the ability to regenerate whole plants from callus or suspension cells by transfer to a modified culture medium. The modifications primarily responsible for triggering plant regeneration involve the concentrations and kinds of plant hormones used in the medium. Three steps involved in plant tissue culture are as follows:

- Initiate independent single cell growth from plant tissues.
- Grow these undifferentiated cells for several generations in a liquid culture medium, and
- Subsequently regenerate whole plants from the cultured cells.

This cycle can be repeated as often as desired.

By changing the content of the growth medium in Step 2 above, the experimenter can select cells with new properties. In this way, tissue culture may provide plants that are disease resistant, stress tolerant, virus free, or nutritionally self-sufficient. Using tissue culture methods, genetic variability in cells can be increased. New breeding systems, that require just a fraction of the developmental time usually needed for conventional breeding, are possible.

REGULATORY BACKGROUND

Any precedent for regulating the agricultural genetic engineering industry will probably be based on the National Institutes of Health (NIH) guidelines for recombinant DNA research which are detailed in the Federal Register (NIH, November 21, 1980). The guidelines were first published in June, 1976 and are periodically updated. Additions and amendments are reviewed and recommended by the NIH Recombinant Advisory Committee (RAC) which consists of nongovernment members with expertise in scientific fields relevant to recombinant DNA technology and biological safety. In addition, the U.S. Department of Agriculture (USDA) has an ad hoc recombinant DNA committee, which submits its recommendations to NIH. In general, current containment levels are considered too restrictive for most plant research, and most recommendations have been for lower containment levels.

All institutions engaged in recombinant DNA research must establish an Institutional Biosafety Committee (IBC) to review projects for compliance with the NIH guidelines and for adoption of emergency plans covering accidental spills and contamination of personnel. Investigators are required to notify their IBC prior to initiation of experiments. The IBCs are responsible for reviewing the registration documents for consistency with provisions of the guidelines and for advising the investigators on necessary changes. IBCs and investigators are still required to petition NIH's Office of Recombinant DNA Research directly about experiments that are not explicitly covered by the guidelines, that require case-by-case review, or that involve exceptions to the provisions.

NIH Physical and Biological Containment Requirements

Two types of containment are used in recombinant DNA research: (1) physical containment, which involves special procedures, equipment, and

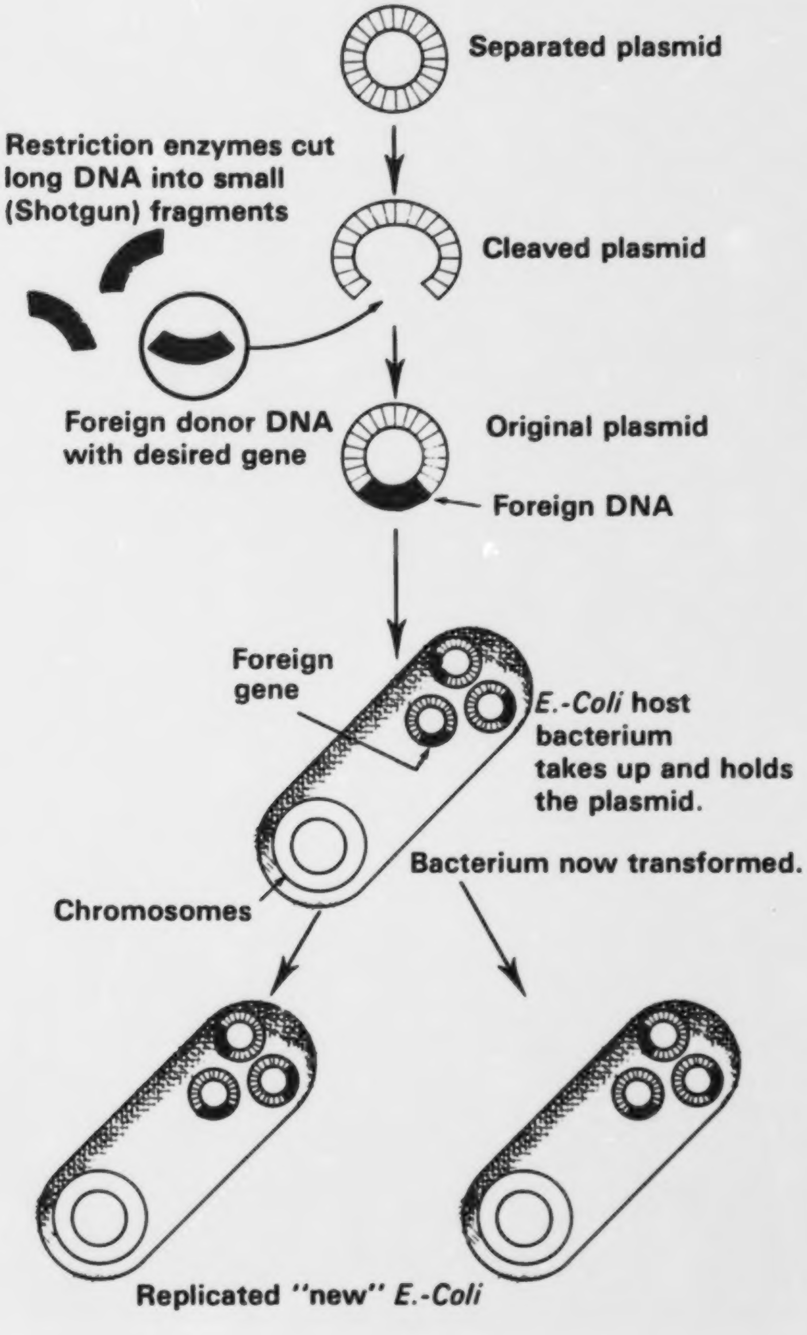
Separated plasmid
Restriction enzymes cut long DNA into small (Shotgun) fragments
Cleaved plasmid
Foreign donor DNA with desired gene
Original plasmid
Foreign DNA
Foreign gene
E.-Coli host bacterium takes up and holds the plasmid.
Bacterium now transformed.
Chromosomes
Replicated "new" *E.-Coli*

Figure 1. Cloning: The mechanics of recombinant DNA.

laboratory installations that provide physical barriers, and (2) biological containment, which limits either the infectivity of a vector or vehicle (plasmid or virus) for specific hosts or its dissemination and survival in the environment. Different levels of containment, appropriate for experiments with different recombinants, can be established by applying various combinations of physical and biological barriers, along with the constant use of standard procedures. The various physical and biological containment levels are described briefly in Table 2.

Containment Requirements for Agricultural Genetic Engineering Experiments

Containment for Genes--

In shotgun experiments, genes from a number of free-living bacteria including *Azotobacter*, *Aspirillum*, *Rhodospirillum*, *Alcaligenes*, *Pseudomonas*, and *Escherichia* and from the symbiotic bacterium, *Rhizobium*, can be cloned and inserted into higher plant cells under relatively low containment requirements (HV1 P2, HV2 P1) (Table 3). The formation of DNA recombinants from cellular DNAs that have been purified can be carried out under lower containment conditions because there is little possibility that a modified virulent organism could escape from the laboratory. All the above organisms are Class 1 (low- or no-hazard) organisms (Office of Biosafety, HEW, 1974).

Containment conditions are higher for *Klebsiella*, *Clostridium*, and *Salmonella* (HV2 P3) because they are known pathogens. Several species of *Clostridium* and all species and serotypes of *Klebsiella* and *Salmonella* are Class 2 (low-hazard) organisms (Office of Biosafety, HEW, 1974). *Clostridium* is commonly found in the intestinal tracts of man and other animals. Two species are known to cause botulism and tetanus. *Klebsiella* inhabits the human intestinal, respiratory, and urogenital tracts. One species causes pneumonia. All known forms of *Salmonella* are pathogenic to man and/or other animals, causing diseases such as typhoid fever, gastroenteritis, and enteric fever. One species of *Pseudomonas*, *P. pseudomallei*, is a Class 3 (moderate-hazard) organism (Office of Biosafety, HEW, 1974), that causes melioidosis or fatal blood poisoning. About ninety species of *Pseudomonas* are plant pathogens that cause leaf spot, leaf stripe, wilt, and similar diseases. Containment requirements for *P. pseudomallei* are EK1 P3, while requirements for the other species are HV1 P2. A proposal allowing DNA recombination experiments with Class 3 organisms and certain plant pathogens has been accepted (NIH, July 29, 1980). Formation of DNA recombinants using DNA from *Klebsiella*, *Clostridium*, *Salmonella*, or *Pseudomonas* that has been purified and in which the absence of harmful sequences has been established can be carried out under containment conditions lower than P3. Experiments with Class 4 and Class 5 organisms are prohibited.

Genes from higher plant cells and plant viruses can be cloned under low containment requirements (HV1 P2 or HV2 P1). If a plant source makes a potent polypeptide toxin, containment is raised to HV2 P3. If a nonpolypeptide toxin is made, HV1 P3 or HV2 P2 is necessary. In shotgun experiments, genes for the toxin might be transmitted by mistake. A polypeptide toxin would be controlled by a relatively simple DNA sequence

TABLE 2. DESCRIPTIONS OF PHYSICAL AND BIOLOGICAL CONTAINMENT REQUIREMENTS

Category or Level of Containment	Description
	Physical Containment
P1	No special containment or laboratory design is required. All biological wastes shall be decontaminated.
P2	Biological safety cabinets shall be used to contain aerosol-producing equipment. Laboratory admittance is restricted. All biological wastes shall be steam sterilized before disposal.
P3	Biological safety cabinets shall be used to contain aerosol-producing equipment. Laboratory admittance is restricted and through a controlled access area. Controlled air movement in the laboratory is required. All biological wastes shall be steam sterilized before disposal.
P4	Experiments must be conducted in Class III cabinets or in Class I or II cabinets in which personnel are required to wear one-piece positive-pressure isolation suits. The area should be under negative pressure and the exhaust air filtered. Laboratory admittance is restricted and is through a clothing exchange and shower area. Laboratory clothing must be completely exchanged on each exit. The laboratory must have an individual air supply and exhaust system. Exhaust air will be HEPA filtered. An individual vacuum system with in-line HEPA filters is required. Water supply, liquid, and gaseous service shall be protected by backflow-prevention devices. All waste must be steam sterilized or decontaminated by suitable means before removal from the P4 facility.

TABLE 2. (Continued)

Category or Level of Containment	Description
	Biological Containment
HV1	Moderate level of containment. Includes *E. coli* K-12 or other prokaryotes comparable in containment.
HV2	High level of containment. Chances of the escape of recombinant DNA either via survival of recombinant organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$.
HV3	Highest level of containment. All HV2 criteria are met. In addition, containment must be independently confirmed by tests in animals, and the relevant genotypic and phenotypic traits independently confirmed.

TABLE 3. CONTAINMENT REQUIREMENTS FOR VARIOUS GENES USING CERTAIN HV1 AND HV2 SYSTEMS(a)

Genes	Source	Containment(b)	NIH Guideline Section
Nitrogen fixation (nif)	*Rhizobium*	HV1 P2	III-A-1-b
	Klebsiella	HV2 P3(c)	III-A-1-b
	Azotobacter	HV1 P2	III-A-1-b
	Clostridium	HV2 P3(d)	III-A-1-b
	Aspirillum	HV1 P2	III-A-1-b
	Rhodospirillum	HV1 P2	III-A-1-b
Hydrogen uptake (hup)	*Alcaligenes*	HV1 P2	III-A-1-b
	Rhizobium	HV1 P2	III-A-1-b
Photosynthesis (cfx)	*Alcaligenes*	HV1 P2	III-A-1-b
	Higher plants	HV1 P2 or HV2 P1(e)	III-A-2-b
Water-splitting (lit)	Blue-green algae	HV1 P2(f)	III-A-1-b
Biological stress (osm)	*Escherichia coli* K-12	EK1 P1	III-0
	Salmonella	HV2 P3(c)	II-A-1-b
	Higher plants	HV1 P2 or HV2 P1(e)	III-A-2-b
Cellulose utilization (cut)	Microorganisms	HV1 P2	III-A-1-b
Denitrification (den)	*Klebsiella*	HV2 P3(c)	III-A-1-b
	Pseudomonas	HV1 P2(c)(g)	III-A-1-b
Plant viruses		HV1 P1	III-A-2-a

(a) Source: NIH, November 21, 1980.

(b) The formation of DNA recombinants from cellular DNAs that have been purified and in which the absence of harmful sequences has been established can be carried out under lower containment conditions than those used for the corresponding shotgun experiment (III-0).

(c) If DNA segments are entirely from *Klebsiella*, *Salmonella*, or *Pseudomonas* and propagation is within these organisms only, experiments are exempt from these guidelines (Appendix A of NIH Guidelines).

(d) HV2 P3 is required for *Clostridium botulinum*, *C. chauvoei*, *C. novyi*, *C. haemolyticum*, *C. histolyticum*, *C. septicum*, and *C. tetani*. All others require only HV1 P2.

(e) If a plant source makes a potent polypeptide toxin, containment is raised to HV2 P3. If a nonpolypeptide toxin is made, HV1 P3 or HV2 P2 is necessary.

(f) Cloned segments of *Anabaena* DNA may be transferred into *Klebsiella* under P2 physical conditions (Appendix E of NIH Guidelines).

(g) EK1 P3 is required for *P. pseudomallei*. Others require only HV1 P2.

(probably one gene encoding the toxin). A nonpolypeptide toxin, on the other hand, would be controlled by a more complex set of DNA sequences and would probably not be the product of one gene. As previously noted, if purified DNA is used and the absence of harmful sequences has been established, lower containment conditions can be employed.

Containment for Vectors--

Containment for the use of *Agrobacterium* as a vector is relatively high (P3) (Table 4) because it is a highly virulent plant pathogen that produces crown gall disease. *Agrobacterium* has a worldwide distribution and a wide host range. Its virulence is one of the main reasons for its use as a vector. The escape of *Agrobacterium* carrying new genes might have wide-ranging environmental consequences. Recently, however, some *Agrobacterium* experiments have been permitted at lower containment levels (NIH, March 12, 1981; November 21, 1980). While *Agrobacterium* is a virulent plant pathogen, plant tissue must be injured to permit infection. The genetic information introduced into plant cells by the *Agrobacterium tumefaciens* Ti plasmid is not transmitted at meiosis into seeds. DNA from plants and nonpathogenic prokaryotes may be cloned under P2 containment conditions. Transfer into plant parts or cells is permitted at the same containment level used for cloning in *Agrobacterium*. Containment levels have been reduced to P1 for well characterized genes, such as those that encode yeast alcohol dehydrogenase and maize zein.

Plant virus vectors, such as cauliflower mosaic virus and bean golden mosaic virus, have narrow host ranges and are relatively difficult to transmit mechanically to plants. Since they are unlikely to be accidentally transmitted by spillage of purified virus preparations, containment requirements are low (P1) (Table 4). Cauliflower mosaic virus is spread in nature by aphids, in which it survives only a few hours, while bean golden mosaic virus is spread by whiteflies. Neither is transmissible by seed or pollen.

Organelles, plasmids, and chromosomal DNAs used as vectors have a slightly higher containment requirement (P2 or P1) (Table 4) because there is a small possibility that DNA transmitted by such vectors could escape through pollen, seeds, or other propagules.

E. coli K12 has a low containment requirement (P1) (Table 4) because it is thoroughly adapted for use in the laboratory and has only a small chance of survival in nature. A proposal to include *Saccharomyces cerevisiae* for P1 containment was recently passed (NIH, July 29, 1980) since it also has only a small chance of survival in nature. However, containment should be modified to prevent spread of pollen, seeds, or other propagules if this is a possible escape route.

Rhizobium has not yet been proposed for use as a vector in any DNA recombination experiments. *Rhizobium* probably will require only a P1 containment (W. Gartland, personal communication). The host range of *Rhizobium* is limited to members of the Leguminosae.

TABLE 4. CONTAINMENT REQUIREMENTS FOR VARIOUS TYPES OF HOST VECTORS(a)

	Host		
Vectors	Intact Plant	Tissue Culture Plants in Axenic Culture	NIH Guideline Section
Agrobacterium	P3(b)	P3(b)	Appendix E
Plant Viruses			
Cauliflower Mosaic Virus	P1	no containment(c)	III-C-3
Bean Golden Mosaic Virus			
Organelle, plasmid, or chromosomal DNAs(d)	P2(P1)(e)	P1	III-C-4
Escherichia coli K-12	P1(f)	P1	III-C-6
Saccharomyces cerevisiae	P1(f)	P1	(g)
Rhizobium	P1	P1	(h)

(a) Source: NIH, November 21, 1980.

(b) Cloned DNA fragments may be transferred to *A. tumefaciens* using a nonconjugative *E. coli* plasmid vector and into plant parts or cells in culture under containment conditions one step higher than required for the desired DNA in HVI systems (Appendix E). However, DNA from plants and non-pathogenic prokaryotes may be cloned and transferred into plant parts or cells in culture under P2 containment. NIH recently granted permission to Mary Dell-Chilton, Washington University, to use *Agrobacterium* to transfer two well characterized genes (yeast alcohol dehydrogenase and maize zein) to tobacco plants using P1 containment (National Institutes of Health, March 12, 1981). A proposal by Donald J. Merlo, University of Missouri, to introduce genes cloned in *E. coli* K-12 into *Arabidopsis* plants using *Agrobacterium tumefaciens* carrying an *E. coli*/Ti hybrid plasmid vector using P1 containment conditions was also recently accepted (NIH, November 21, 1980.).

(c) Experiments using plant virus genomes to propagate DNA sequences from eukaryotic viruses require containment (See Table 2).

(d) Containment is required only when DNA recombinants are formed between vectors and DNA from cells other than host species. When DNA recombinants are formed between such vectors and host DNA and are propagated only in the host (or a closely related strain of the same species), experiments are exempt from the guidelines.

(e) P1 containment should be used if plants are too large for P2 facilities. But, run-off water should be sterilized if this is a plausible route for secondary infection, and standard P2 practices should be used for microbiological procedures.

(f) Containment should be modified to prevent the spread of pollen, seeds, and other propagules. A request by Ron Davis, Stanford University, to field test corn plants into which corn recombinant DNA has been added using *E. coli* or *S. cerevisiae* vectors is still pending.

(g) A recent proposal to include *S. cerevisiae* with *E. coli* for P1 containment was passed (NIH, July 20, 1980).

(h) At this time, no research has been proposed using *Rhizobium* as a vector, so it is not included in the Guidelines. However, NIH officials state that it would probably be assigned a P1 containment level (W. Gartland, personal communication).

Containment for Hosts--

Cells in culture or plants growing in axenic culture as the host species generally require lower containment than intact plants because of the fragility of such hosts and the low survival rate of cultured cells outside the laboratory.

Generally, P1 or P2 containment is required for intact plant hosts. P1 conditions include a limited-access greenhouse or growth chamber that is insect restrictive, preferably made so with positive air pressure and with an insect-fumigation regime. In systems where propagules should be contained or where infectious agents which generate airborne propagules are used, negative air pressure probably is more appropriate. Soil, plant pots, and unwanted infected materials should be removed from the greenhouse or cabinet, sealed in insect-proof containers, and sterilized before disposal. Run-off water should also be sterilized, if this is a potential escape route.

SECTION 2

POTENTIAL ENVIRONMENTAL BENEFITS AND HAZARDS OF AGRICULTURAL GENETIC ENGINEERING

In this section, the scope of impacts of agricultural genetic engineering on the environment and public health is defined and discussed. Physiological and biochemical background information, as well as a discussion of environmental hazards and benefits, is provided for molecular vectors and genes of potential agronomic importance (Table 5). At the end of this section, a scenario projecting the global impacts of genetic engineering on agriculture in 1990 is presented.

AGROBACTERIUM (Ti PLASMIDS) AND PLANT VIRUSES - GENE VECTORS FOR AGRICULTURAL GENETIC ENGINEERING

Several vectors for transferring foreign genes into higher plant cells or tissues are currently under development. These include *Agrobacterium tumefaciens*, caulimoviruses (e.g., cauliflower mosaic virus), geminiviruses (e.g., bean golden mosaic virus), potato leafroll virus, and liposomes. Below we assess the current data on *Agrobacterium* and plant DNA viruses as they relate to transfer of genes to plants and to hazards or benefits derived from dispersion of new genomes in the environment. Since liposomes are artificially constructed membranous vesicles, they cannot grow or divide, and therefore they do not pose a threat to the environment or to human health.

Background

Agrobacteria--

Agrobacterium tumefaciens is a gram-negative, soil bacterium that causes a neoplastic disease of dicotyledonous plants called crown gall (Braun and White, 1943). Crown gall tumors can proliferate autonomously in tissue culture and, in a few cases, tumorous plants can be regenerated from the cells (Braun and Wood, 1976). The tumorous state of crown gall tissue results from the transfer of DNA from *A. tumefaciens* to the plant cell (Schell et al., 1979). This natural system for DNA exchange is being studied extensively. In addition to providing an interesting system of genetic exchange and tumor induction, *Agrobacterium* may make available a vector for the introduction of new genes into plants by molecular genetic engineering methods.

Both physical and genetic evidence indicates that the agents responsible for crown gall tumor induction are large plasmids, pTi (tumor-inducing plasmids), contained in all oncogenic strains of *A. tumefaciens*

TABLE 5. GENES AND GENE FUNCTIONS WHOSE ECOLOGICAL AND SOCIAL-ECONOMIC IMPACTS WERE ASSESSED[(a)]

Genes	Source of Genes	Potential Significance
Gene transfer vectors	*Agrobacterium tumefaciens*/ Plant DNA viruses	Insertion of new genes into crop plants
Nitrogen fixation		
nif	*Klebsiella*/*Rhizobium*	Genetic engineering of new nitrogen-fixing plants and microbes
hup	*Alcaligenes*/*Rhizobium*	Enhancing energy efficiency of symbiotic nitrogen fixation in legumes
lit	Blue-green algae	Key genes for production of reductant during photosynthesis
Denitrification		
den	*Klebsiella*/*Pseudomonas*	Important in soil fertility and water quality
Physiological stress		
osm	*E. coli*/*Salmonella*/ higher plants	Osmoregulatory genes provide tolerance for drought, salt, and thermal stresses in microbes and plants
Photosynthesis		
cfx	*Alcaligenes*/higher plants	Enhancing efficiency of photosynthetic carbon dioxide fixation in plants

(a) The nif genes are clustered in an operon and are conveniently referred to as genes. However, the other genes listed; hup, lit, den, osm, and cfx; are all only short names for gene functions. They *may* involve many different genes which are not tightly linked.

(Zaenen et al., 1974). Loss of these large plasmids results in loss of bacterial oncogenicity, and introduction of a Ti plasmid into originally nononcogenic, plasmid-free *Agrobacterium* strains render them oncogenic.

The Ti plasmids of tumor-inducing strains of *A. tumefaciens* are large, ranging in size from 150 to more than 200 kilobase pairs (Depicker et al., 1978). These plasmids encode functions for oncogenicity, opine (arginine derivatives) biosynthesis and catabolism, conjugative transfer between bacteria, sensitivity to antibiotics, and exclusion of bacteriophage Apl (Holsters et al., 1980). Within the Ti plasmids is a special DNA segment called T-DNA, which can stably integrate into the nuclear genome of transformed plant cells (Dell-Chilton et al., 1977; Yadav et al., 1980).

Agrobacteria, and possibly other bacteria, have evolved this intricate plasmid mechanism to transfer specific genetic information to plants in such a way that the transformed plant cells express a number of new phenotypes, such as uncontrolled proliferation and the synthesis and probable release of opines. The bacteria, but not the plant, benefit from this transformation because they can selectively utilize the opines for their own growth and proliferation. The surface of crown galls therefore provide an ecological niche in which those bacteria that harbor plasmids with genes which enable the use of opines for energy, carbon, and/or nitrogen source have an important selective advantage over other soil bacteria.

Plant DNA Viruses--

The simple genomes of specific plant DNA viruses may have some advantages not offered by other types of genetic vectors. Of the several hundred known plant viruses, only two or three have DNA as the genomic material. The others are RNA viruses. The plant DNA viruses are the caulimoviruses, the geminiviruses, and the potato leafroll virus. Little is known about the potato leafroll virus because it is present in only very small quantities in infected plants. Therefore, it will not be discussed further here. The caulimoviruses encompass at least six individual viruses with double-stranded DNA: cauliflower mosaic virus, carnation etched ring virus, dahlia mosaic virus, mirabilis mosaic virus, figwort mosaic virus, and strawberry vein banding virus (Shepherd, 1979). Of these, cauliflower mosaic virus is the most thoroughly studied (even though the data base is still relatively small) and is being seriously examined as a cloning vector for transferring genetic information to plants (Szeto et al., 1977; Lebeurier et al., 1980). Cauliflower mosaic virus infects species of the Cruciferae (e.g., cabbage, cauliflower, mustard), and some strains infect members of the Solanaceae (e.g., tobacco and carrot) (Broadbent, 1957; Lung and Pirone, 1972). The genome of cauliflower mosaic virus is a double-stranded, circular DNA molecule of about 7 kilobase pairs (Shepherd et al., 1970). A physical map of the cauliflower mosaic virus genome based on cleavage by restriction endonucleases has been determined (Meagher et al., 1977), and the viral genome can be clonally replicated in *E. coli* (Szeto et al., 1977).

Geminiviruses are a group of single-stranded DNA viruses including bean golden mosaic virus, beet curly top virus, cassava latent virus, *Euphorbia* mosaic virus, maize streak virus, and tobacco leaf curl virus. Of these, bean golden mosaic virus is the most studied. This virus infects members of the Leguminosae, such as Phaseolus vulgaris and P. lunatus. As compared with the caulimoviruses, geminiviruses have smaller and less complex particles with single-stranded DNA genomes that may be multipartite in nature.

Compared to Agrobacterium, the plant viruses are less well developed as vectors for recombinant DNA experimentation. Information is lacking on essential virus-coded protein products and their locations on the genome. So, insertions of foreign DNA into a viral genome may destroy the ability of the foreign gene to be expressed or to be replicated. Certain plant DNA viruses may be of limited use as vectors. For instance, cauliflower mosaic virus is not transmitted through seed. So only dicotyledonous plants that are not sexually propagated can be subjected to genetic transformation. Also, cauliflower mosaic virus probably requires a helper virus in order to replicate. New genes inserted into the cauliflower mosaic virus genome recombine with the helper virus genome thereby making transduction with a new gene very difficult (R. Meagher, personal communication).

Strategies for Introducing New Genes into Crop Plants--

Two basic approaches are being developed to engineer the genes of plants (Figure 2). These center on utilizing plant DNA viruses or Agrobacterium (the Ti plasmid from Agrobacterium) as vectors (Kado, 1979; Schell et al., 1979).

In the first approach, useful new genes may be joined in vitro to the Ti plasmid, and the resulting hybrid plasmid can then be inserted by transformation into a plasmidless strain of A. tumefaciens. The transformed bacterium inserts the hybrid plasmid (or parts of it) into the plant cell during infection of a whole plant. The infected plant tissues (crown galls) are excised from the plant and are brought into tissue culture. These tissue cultures are then encouraged to differentiate into whole, seed-bearing plants.

In the second approach, individual plant cells or protoplasts are transduced by direct insertion of a hybrid plasmid using liposomes or by employing DNA plant viruses, containing new genes. The protoplasts, now housing the new genes, can then be regenerated into whole plants that will produce seed.

Although both approaches are logical, they are clearly oversimplifications and many problems remain. Many genetic and biochemical barriers in plants (some yet to be identified) must be surmounted before genetic engineering can be successful (Kado, 1980). Even though the techniques for maintaining crown gall tissues in axenic culture are well developed, the regeneration of the tumorous tissues into whole plants is less well established. In fact, knowledge about the regeneration process has been a major limiting factor in developing most normal plant cell or protoplast cultures (Thorpe, 1978). Also, pieces of Ti plasmids found in plants derived from tumorous tissues are lost during seed production (Yang et al., 1980) so that the feasibility of generating seed stock of genetically modified crops is greatly reduced. This could mean that the use of Ti plasmids for genetic engineering of plants will have to be limited to plant species that can be reproduced by vegetative propagation. Another limitation is posed by the

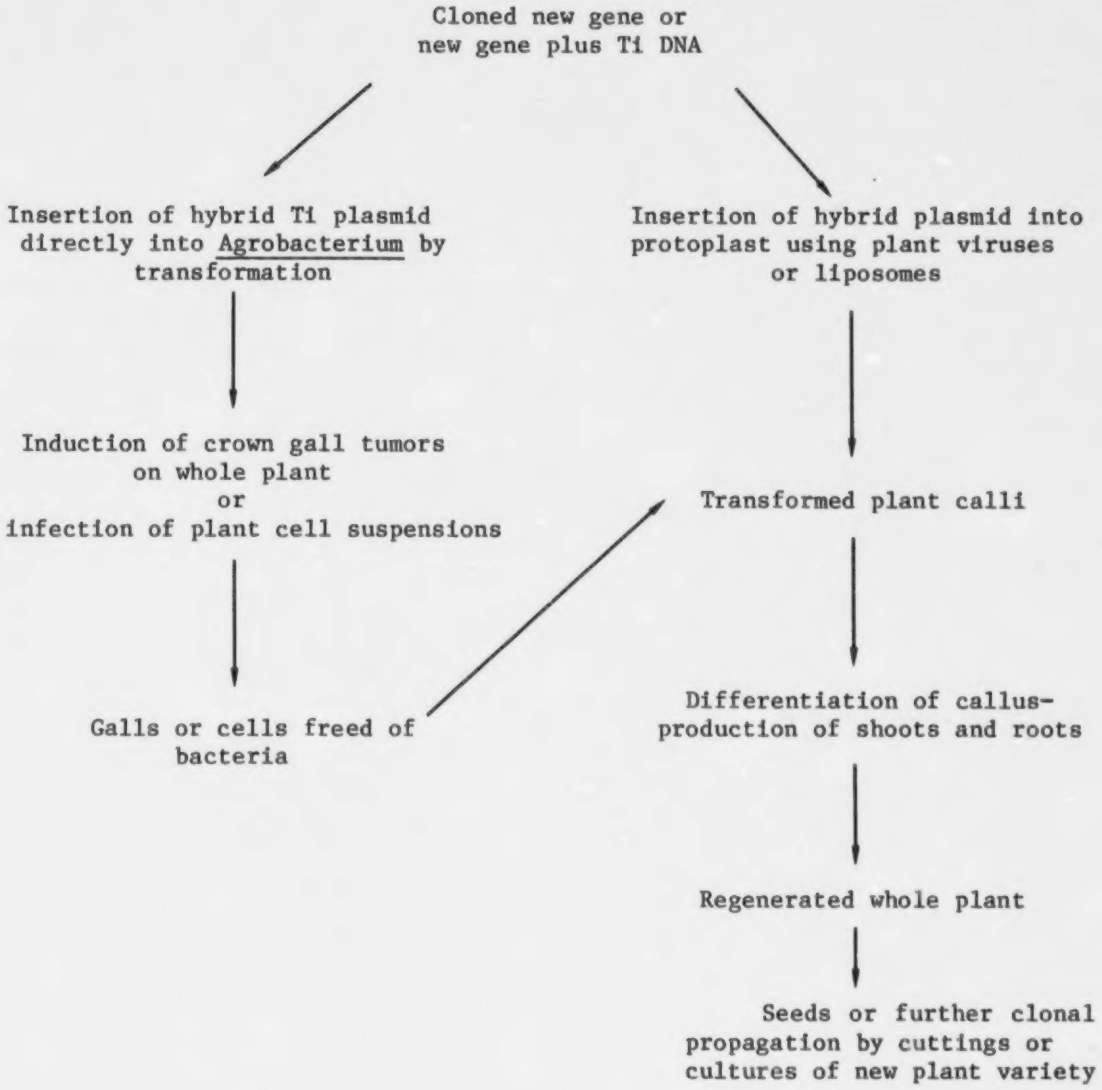
Cloned new gene or new gene plus Ti DNA
Insertion of hybrid Ti plasmid directly into Agrobacterium by transformation
Insertion of hybrid plasmid into protoplast using plant viruses or liposomes
Induction of crown gall tumors on whole plant or infection of plant cell suspensions
Transformed plant calli
Galls or cells freed of bacteria
Differentiation of callus-production of shoots and roots
Regenerated whole plant
Seeds or further clonal propagation by cuttings or cultures of new plant variety

Figure 2. Flow diagram of two strategies for introducing foreign genes into higher plants.

fact that A. tumefaciens and most of the plant DNA viruses rarely infect monocotyledonous plants. Many of our most important crop plants, including corn, wheat, and rice, are monocotyledons. However, many vegetable crops can potentially be engineered with Agrobacterium and plant DNA viruses.

Potential Environmental Benefits and Hazards

Agrobacteria--

Several features of Agrobacterium could promote the spread of pTi DNA throughout the environment. The Ti plasmids are conjugative in that they carry genes that enhance conjugation and transfer of Ti plasmids between donor and acceptor bacteria (Kerr et al., 1977). Observations suggest that a conjugative mechanism might be involved also in the transfer of the Ti plasmid from bacteria to plant cells (Lippincott and Lippincott, 1975). The host range for Ti plasmids has been further extended by production of hybrid plasmids constructed by combining the Ti plasmid with the wide-range plasmid RP4. The Ti-RP4 plasmid can be transferred by conjugation at a high frequency to many other gram-negative bacteria, such as Pseudomonas, Rhizobium, and Escherichia (Schell and Van Montagu, 1978).

The promiscuity of Ti plasmids may be considered both an advantage and a risk. These plasmids offer chances to study the natural mechanism of DNA transfer among different organisms such as bacteria and plants, to examine the regulatory mechanisms involved in bacterial conjugation, and to understand the molecular biology of neoplastic transformation. On the other hand, plasmids that confer increased capabilities to Agrobacterium may be more easily passed to other Agrobacteria species (and other gram-negative bacteria). For instance, plasmid transfer may produce more virulent strains of crown-gall, hairy root (White and Nester, 1980), and other plant diseases.

Given the risks, containment levels for experiments involving recombinant DNA which increases the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange are determined by NIH on a case by case basis. In April 1980, NIH ruled specifically on the use of A. tumefaciens as a host-vector system. According to that ruling, cloned DNA fragments from a nonprohibited source can be transferred into A. tumefaciens (containing a Ti plasmid) using a nonconjugative E. coli plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of DNA replication of an Agrobacterium plasmid. Such gene transfers can be conducted under containment conditions one step higher than would be required for the desired DNA in HVI systems. Transfer into plants or cells in culture would also be permitted at this higher containment level.

Several test decisions involving the NIH ruling about the genetic engineering of plant pathogens (e.g., Agrobacterium) have been made. For instance, it was agreed that a hybrid plasmid composed of (1) E. coli plasmid pBR325, (2) the origin of replication and transfer genes of A. tumefaciens plasmid Ti, (3) the thiamine gene of E. coli, and (4) Arabidopsis DNA, may be transformed into A. tumefaciens under P1 conditions. A. tumefaciens may subsequently be used to introduce the composite plasmid carrying Arabidopsis DNA and the E. coli thiamine gene into Arabidopsis plants under P1 containment conditions. Research with this hybrid plasmid is to be carried out by

Dr. Donald Merlo at the University of Missouri, Columbia. In another case decision, the use of A. tumefaciens with the hybrid plasmid Ti-RP4 was considered as a HV1 system under P3 containment. Finally, in another instance, NIH has approved experiments involved in testing mutants of A. tumefaciens on carrot slices. The DNA of Agrobacterium and an EK2 vector may be cloned back into Agrobacterium, and these transformed bacteria may then be introduced into plant cells in culture under P1 conditions.

NIH has also ruled on using nonpathogenic vectors for transferring DNA to higher plants. DNA from any nonprohibited source, that has been cloned and propagated in E. coli or Saccharomyces under P1 conditions, may be transferred with the E. coli or S. cerevisiae vector used for cloning to any higher plants (angiosperms and gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study. Intact plants or propagative plant parts may be grown under P1 conditions. Containment must be modified to prevent the spread of pollen, seeds, and other propagules. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by the bagging of reproductive structures. Transfers to any other plant will be considered on a case-by-case basis.

Plant DNA Viruses--

Several features of cauliflower mosaic virus make it an excellent candidate for a genetic vector but raise concern about environmental impacts of spreading recombinant viral genomes. The virus is transmissible mechanically to easily propagated herbaceous plants, its free DNA genome (i.e., when separated from the viroid) is infectious (Shepherd et al., 1970), and it has no tissue restriction. In addition, cauliflower mosaic virus is spread by insects and by infested, vegetatively propagated, planting material. Sucking insects, called aphids, are its only natural vectors. Feeding aphids pick up the viruses on their mouthparts and carry them to healthy plants during later feeding. Some aphid species can transmit viruses for several hours and occasionally for 1 or 2 days after acquisition. Aphids are common in greenhouses where unintentional transmissions may occur frequently. This problem can be prevented by use of nonaphid-transmitted strains of cauliflower mosaic virus. Transmission of this virus has been thoroughly reviewed (Shepherd, 1976).

One feature of geminiviruses that make them good candidates for genetic vectors is their wide host range. Geminiviruses infect important crop plants in the Leguminosae and Gramineae families. The beet curly top virus, for example, can infect 300 species. However, this wide host range also increases the chances of dispersion of undesirable genes. In addition, whiteflies and leafhoppers, that transmit geminiviruses in nature, can maintain the viruses for the lifetime of the insect. The insects may acquire the virus during feeding periods of less than one hour. A brief latent period of 4 to 8 hours is required for the virus to circulate throughout the insect's body. The virus is absorbed through the gut wall into the hemolymph of the insect and is secreted by the salivary glands during feeding. Both whiteflies and leafhoppers are common in greenhouses, so unintentional transmissions may occur (Shepard, 1979).

Plant DNA viruses have several biological characteristics that could make them relatively safe genetic vectors. Cauliflower mosaic virus has a restricted host range (infects plants of the Cruciferae family) and will not infect such important grain crops as corn and wheat. Although the geminiviruses have a wide host range, they are restricted to one tissue, the phloem and are not distributed throughout all somatic tissues. Only one geminivirus, the bean golden mosaic virus, can be mechanically transmitted to plants. Cauliflower mosaic virus can be mechanically transmitted through abrasion of leaf or stem surfaces. However, these DNA viruses are not transmissible by seed or pollen, and for this reason, accidental transmission from spillage of purified virus preparations is unlikely. Also, the fragility of plant protoplasts (into which the virus would be introduced), combined with several properties of the virus, provides adequate safety. Since no apparent risk to the environment from the use of a plant virus/protoplast system is envisioned, NIH has suggested that no special containment is necessary.

When plant DNA viruses are used as vectors in intact plants, or in propagated plant parts, the plant is grown under P1 conditions (i.e., in either a limited access greenhouse or a plant growth cabinet that is insect-restrictive, that preferably is equipped with positive air pressure, and in which an insect fumigation regime is maintained). Soil, plant pots, and unwanted infected materials must be removed from the greenhouse or cabinet in sealed, insect-proof containers and then sterilized. It is not necessary to sterilize run-off water from the infected plants, as this is not a likely route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is necessary.

Infected plant materials that have to be removed from the greenhouse or cabinet for further research must be maintained under insect-restrictive conditions, which provide sufficient containment and which are similar to those required for licensed handling of exotic plant viruses in many countries.

NITROGEN FIXATION (nif) GENES

Background

The biochemical genetics of nitrogen fixation has been recently reviewed by Brill (1980) and Shanmugam et al. (1978). Nitrogen supply is probably one of the most important factors limiting plant growth (NSF, 1980). Even though nitrogen is an abundant atmospheric gas, only certain bacteria and blue-green algae possess the enzyme nitrogenase and are capable of fixing and reducing nitrogen to ammonia. Free-living nitrogen-fixing bacteria (i.e., those that are not intimately associated with a specific plant) include members of *Klebsiella*, *Azotobacter*, *Clostridium*, *Rhodospirillum*, *Aspirillum*, and various cyanobacteria (blue-green algae). Bacteria that normally fix nitrogen only when they are symbiotically associated with a plant are *Rhizobium spp*. (which nodulate legumes) (Vincent, 1974; Fred et al., 1932), certain actinomycetes (which nodulate *Comptonia* and alder) (Torrey, 1978), and *Anabaena azollae* (a cyanobacterium that fixes nitrogen within the leaf pores of the water fern

Azolla) (Peters, 1978). No eukaryotic organisms have been shown to fix nitrogen directly.

The biochemistry of nitrogen fixation is well understood. Nitrogenase is made up of two soluble proteins, called components I and II (Bulen and LeComte, 1966; Eady et al., 1972; Mortenson et al., 1967; Shah and Brill, 1973; Vandercasteele and Burris, 1970). Component I is also known as molybdenum- iron protein or nitrogenase while component II is known as iron protein or nitrogenase reductase (Hageman and Burris, 1978). Substrate binding and reduction take place on component I (Orme-Johnson et al., 1972; Smith et al., 1972; Smith et al., 1973). The role of component II is to supply electrons, one at a time, to component I (Ljones and Burris, 1978; Mortenson et al., 1967).

Since both components are rapidly and irreversibly inactivated by brief exposure to oxygen (Bulen and LeComte, 1966), organisms that fix nitrogen must have different mechanisms to protect their nitrogenases from oxygen. For instance, several nitrogen-fixing bacteria are strict anaerobes. Others, such as Azotobacter, have very high respiratory activity which rapidly lowers the internal oxygen concentration (Phillips and Johnson, 1961). Still others, such as cyanobacteria, package nitrogenase in heterocysts, specialized thick-walled cells that lack the oxygen-producing reactions of photosynthesis (Fleming and Haselkorn, 1973; Peterson and Wolk, 1978; Tel-Or and Stewart, 1977). Symbiotic nitrogen fixers such as Rhizobium protect their nitrogenase from oxygen inhibition by complexing it with the red pigment leghemoglobin, a myoglobin-like protein which can bind oxygen reversibly and with an extremely high affinity. Leghemoglobin is a product of the symbiotic interaction, the globin being produced by the plant, the heme by the bacteria (Beringer et al., 1979). Biological nitrogen fixation, like the industrial process used to produce nitrogen fertilizer (Haber-Bosch process), uses considerable energy. Between 12 and 36 molecules of adenosine triphosphate (ATP) are required for each molecule of nitrogen fixed (Brill, 1979).

The location of the nitrogen fixing (nif) genes has been determined in Klebsiella pneumoniae. The nif operon includes structural genes for the production of the nitrogenase subunits, regulatory genes, and genes for the synthesis of the molybdenum cofactor. Situated in a cluster near the his operon, the nif genes occupy less than 1 percent of the total bacterial chromosome. At least 17 nif genes make up the cluster, and no non-nif genes interrupt it (MacNeil et al., 1978). Complementation analysis with polar mutations (Elmerich et al., 1978; MacNeil et al., 1978; Merrick et al., 1978) and studies of fusions of lac with each gene (MacNeil and Brill, in press) indicate that the genes are organized into seven distinct operons and that the transcription of the operons is in the same direction, namely, toward the his genes (Figure 3).

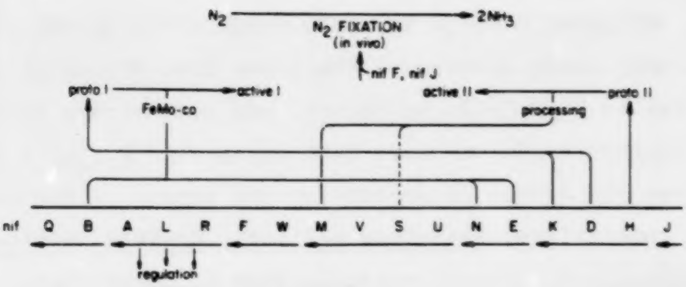

Figure 3. Order, transcriptional organization, and function of the *K. pneumoniae* nif genes. The arrows along the bottom represent the seven individual operons (Source: Brill, 1980).

The nif region is approximately 24 kilobases long (Cannon et al., 1979). A physical map of a large part of the nif region has been produced by restriction endonuclease cleavage analysis of cloned DNA (Riedel et al., 1979). The protein structure of nitrogenase component II is encoded by nif H, but the products of nif M, nif S, and possibly nif V genes are necessary to modify and activate component II (Roberts et al., 1978). Two proteins in component I are specified by nif K and nif D. In order to obtain active component I, an iron molybdenum cofactor (FeMo-co) is required. FeMo-co synthesis requires the products of nif Q, nif B, nif N, and nif E (Roberts et al., 1978). Proteins coded by nif F and nif J genes are necessary to transport electrons to nitrogenase. The electrons break the triple bond between the two nitrogen atoms, converting each atom to a molecule of ammonia (Nieva Gomez et al., 1980; Roberts et al., 1978; Yoch, 1974; Yates, 1971). The activator produced by nif A turns on the production of the nif-coded proteins when the cells lack sufficient fixed nitrogen in their environment. Both oxygen and ammonia repress nitrogen fixation. The function of the nif L product is to prevent nitrogenase synthesis when the cells start aerobic functions. The site between nif L and the promoter for the nif RLA operon at which ammonia regulation occurs is defined as nif R. It is not yet known whether nif R codes for a protein (Roberts et al., 1978; MacNeil and Brill, 1978; St. John et al., 1975). Nif W and nif U are also not essential under normal conditions (Roberts et al., 1978). In *Rhizobium*, some genes for nitrogenase, as well as for many other genes involved in symbiosis and nitrogen fixation, are localized on a plasmid. This was shown recently by hybridization of plasmid DNA with the cloned structural nif genes of *Klebsiella pneumoniae* (Krol et al., 1980).

The nif genes have so far been transferred by the techniques of DNA recombination only among microbes. The genes from *Rhizobium trifolii* have been transferred to *Klebsiella aerogenes*, and preliminary reports of nif gene transfer in photosynthetic microbes have appeared. *E. coli*, *S. typhimurium*, and *K. aerogenes* all accept *K. pneumoniae* nif genes. Other organisms, such as *Agrobacterium tumefaciens*, *Rhizobium meliloti*, *Erwinia herbicola*, and

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Azotobacter vinelandii, accept nif plasmids from K. pneumoniae. However, the new recombinant organisms do not fix nitrogen. Since some of the recipient bacteria do not express the nif genes completely (even if they accept them) while others do not accept them at all, more genes than just the nif cluster obviously are needed for nitrogen fixation, even in bacterial hosts. For example, genes that control the level of free oxygen in the cell are necessary to prevent nitrogenase from being destroyed (Postgate, 1977). Also, genes that regulate the production and activity of nitrogenase and other accessory proteins are required.

The nif gene cluster may have been transferred to plants in nature (Postgate, 1977). However, the simple transfer of nif genes does not confer the ability to fix nitrogen. Nitrogen-fixing bacteria also have the mechanisms for regulating, transcribing, and translating the information on nif and its supporting genes. The means for using this bacterial information will thus have to be introduced along with the nif cluster and whatever else is transferred to plants. The existence of this genetic barrier between prokaryotes and higher plants will make the transfer of nif genes to plants a long-term project (1 to 2 decades). There are several possible mechanisms for transferring this information:

- Plant DNA viruses have sites that can be read by a plant's genetic machinery so these may be useful vectors (see discussion on vectors earlier in this report).

- Some plant organelles (mitochondria and chloroplasts) have more bacterium-like genetic equipment than the main plant chromosomes and, therefore, these organelles might be more satisfactory hosts. It should be noted, however, that mitochondrial codons differ somewhat from the codons used by all other organisms. Other problems with organelles as gene vectors include multiple genome copy number, multiple organelles within a cell, sorting out, and organellar recombination.

- Deliberate construction of more effective symbionts, such as endotrophic mycorrhizae, might be used to effect the transfer of information. Ecological studies are now uncovering more such associations existing naturally in many environments (Postgate, 1977).

Even if nif genes cannot be directly transferred to crop plants, other important advances in enhancing fixation using recombinant DNA techniques may be possible. For example, the regulation of nif can be altered by mutation, so that such free-living nitrogen fixers as A. vinelandii or K. pneumoniae fix nitrogen even when they are supplied with ammonia. These mutants would be derepressed. Partially derepressed mutants might also be obtained that would yield more nitrogen to their hosts (Gordon and Brill, 1972; Gordon et al., 1975). In addition, nitrogen-fixing microbes with only one set of nif genes might be given a second copy of nif genes, which might make them more efficient nitrogen fixers. Another possibility is to increase the length of time that bacteria associated with plants fix nitrogen since these bacteria often stop fixing nitrogen during seed formation. A few more days fixation, which might dramatically increase the protein content of crops, could be induced by genetic manipulation (Postgate, 1977).

Potential Environmental Benefits and Hazards

Modern farming methods have reduced the amount of organic matter in soils to an equilibrium value of 40 to 60 percent of the original amount. Soil fertility (inorganic matter) has deteriorated as well. Crop yields can only be maintained only through the continued application of energy-intensive, chemical fertilizers.

Crop productivity (yield per unit land area) during the last quarter century is highly correlated with the input of nitrogen fertilizer. For example, the yields of cereal grains in both industrialized and less developed countries from 1950 to 1975 paralleled the increasing rates at which fertilizer has been applied (Hardy et al., 1975). Up to one-half of the 3 percent average annual increase in world grain production was due to the exponential increase in the use of nitrogen fertilizer. U.S. fertilizer use during this 25 year period increased by a factor of 2.7, and nitrogen use increased by a factor of approximately 10 (Paul et al., 1977).

From data supplied by the USDA (USDA, 1980), projections of nitrogen fertilizer use in 1990 have been made. On a worldwide basis, 1.4 times as much fertilizer will be consumed in 1990 as in the mid-seventies. Usage in the developing countries is expected to increase by a factor of 1.7, in the developed countries by 1.2, and in the centrally planned countries by 1.6.

Environmental effects of this increased use of fertilizer include greater depletion of the ozone layer by the nitrous oxide in fertilizer, increased eutrophication in aquatic environments by leaching of applied fertilizer into watercourses, lakes, and reservoirs, and the contamination of drinking water supplies with nitrates, which could increase the incidence of methemoglobinemia, a disease which affects primarily infants under the age of three.

The enhancement of biological nitrogen fixation by recombinant DNA techniques could result in increased crop productivity without increased use of fertilizer and without the detrimental effects noted above. In addition, energy and dollar savings in industrial nitrogen production (Haber-Bosch process) and transportation would occur. Approximately $10 billion are spent annually on chemical fertilizers in the U.S. alone (Chemical Week, 1980). If nitrogen was not a limiting factor, other plant nutrients such as potassium, sulphur, magnesium, and phosphorus would become limiting to plant growth. If, by proper husbandry, these were attended to then the rate of carbon dioxide fixation would become limiting, as it is today with otherwise well-fertilized legumes. Techniques of genetic engineering may provide solutions to this limitation (also see section below on carboxylase genes, below) (Postgate, 1977).

Detrimental effects of enhancing nitrogen fixation using recombinant DNA techniques can be envisioned, but they are slight compared to the potential benefits. Any plant or microbial system that fixes nitrogen must consume more ATP than it does when ammonia fertilizers are used. No fewer than 7 to 8 ATP molecules would be required per molecule of ammonia received by the plant, and the number could be greater if a large amount of photosynthate had to be expended (and therefore ATP lost) to protect nitrogenase from oxygen. Under identical environmental conditions, a nitrogen-fixing cereal would probably not be as productive as one supplied with comparable amounts of ammonia. One consequence of this increased energy need might be that the range of a crop might move towards the equator, but by what distance is difficult to judge. In any event, farmers might find the lower yields from nitrogen-fixing crops acceptable to the extent that reduced income was more than compensated by lower inputs (and costs) of nitrogen fertilizer.

If the property of nitrogen fixation became more widespread in wild plants, more vigorous growth of weeds and natural grasses might become a problem. This seems unlikely for two reasons. First, most common crop plants are self-pollinated. Crossing with related weed species occurs less than 1 percent of the time (D. Ray, personal communication). Second, growth of nitrogen-fixing weeds would only be more vigorous in seriously nitrogen-deficient soils, an unlikely condition under modern agricultural practice.

Transfer of nitrogen fixation to plant pathogens is another potentially harmful possibility. For example, if the plant-rotting microbe Erwinia or its host plants developed nitrogen fixing ability, pathogenicity would be increased because plants low in nitrogen are usually more resistant to Erwinia than plants with high levels of nitrogen (Postgate, 1977). However, the major factor that usually influences plant susceptibility to Erwinia attack is moisture, not nutrition (D. Coplin, personal communication).

As nitrogen is often a limiting nutrient for decomposition of soil organic matter, increased nitrogen fixation by plants could eventually lead to reduction of soil organic matter, both degrading soils and releasing more carbon dioxide into the atmosphere (Barney, 1980).

One can speculate on the impact is transfer of nitrogen fixation to the E. coli of a ruminant animal. This probably would have no effect because the rumen contains repressive amounts of ammonia, and the limited amount of air in the rumen would probably limit the quantities of nitrogen fixed. Derepressed nif (which is not repressed by ammonia) might yield nitrogen-fixing animals that could live on cellulose substrates without added protein (Postgate, 1974). It might also kill the animals through ammonia toxicity (Postgate, 1977). This possibility seems very remote since weakened strains of E. coli are used in recombinant DNA research. Ammonia toxicity caused by overfeeding of protein is more of a problem (R. Conrad, personal communication).

HYDROGEN UPTAKE (hup) GENES

Background

During nitrogen fixation, some of the electrons destined for nitrogenase do not combine with nitrogen gas to form ammonia but instead react with H^+

ions to form hydrogen gas. This process consumes ATP. Some bacteria have a mechanism to recover some of the electrons and thereby reduce the ATP lost by hydrogen evolution. These bacteria contain the enzyme hydrogenase which oxidizes hydrogen and yields either H^+ ions or electrons capable of reducing nitrogenase or energy in the form of ATP to drive nitrogen fixation (Figure 4) (Brill, 1979).

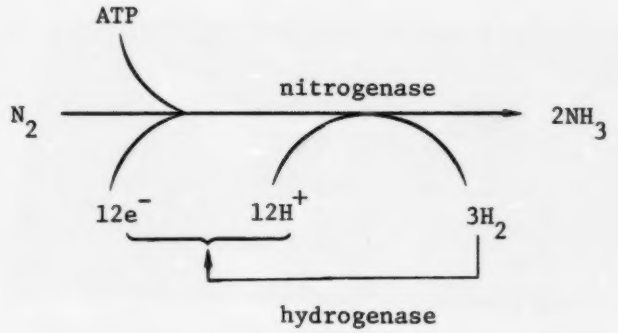

Figure 4. Nitrogen fixation and the hydrogen uptake mechanism. (Source: Brill, 1979).

Several agronomically important species of *Rhizobium* do not have an active hydrogen uptake system (Schubert and Evans, 1976; Lim, 1978). In a recent study conducted at 70 different locations in the major areas of soybean production, about 75 percent of all *Rhizobium japonicum* strains tested lacked hup activity (Lim et al., 1980). One of the easiest ways to enhance nitrogen fixation by legumes would be to introduce hydrogen uptake (hup) genes for the enzyme hydrogenase or other genetic systems for hydrogen uptake mechanisms into the *Rhizobium* strains that lack them. Hup genes is used simply to refer to genes which code for hydrogen uptake mechanisms. This process may be the product of a single gene or, more likely, multiple genes which may or may not be tightly linked.

Hydrogen bacteria, which use hydrogen as an energy source, may serve as a convenient source of hup genes. The hydrogen uptake mechanisms in these bacteria is essentially the same as that shown in Figure 4. Large plasmids that determine the ability to metabolize hydrogen in several species of hydrogen bacteria have been identified. For instance, the hup genes have been transferred from *Norcardia opaca* to *Norcardia erythropolis* by a natural conjugation system; the capacity was lost after exposure to mitomycin (Schubert and Evans, 1977). This suggests that the genes are situated on a transferable plasmid. Six other strains of bacteria (three strains of *Alcaligenes eutrophus*, *A. paradoxus*, *P. facilis*, and *P. palleronii*) also lost their ability to use hydrogen as an energy source after exposure to mitomycin. The authors concluded that the *A. eutrophus* strains, and probably the other species of hydrogen bacteria, contained a large plasmid required for hydrogen metabolism and autotrophic growth (Lim et al., 1980). Plasmids have been detected in several strains of *R. japonicum*. So far there is no correlation between presence of the plasmid and the hydrogen uptake ability in R. japonicum. Further characterizations and modifications of plasmids from hydrogen uptake bacteria may allow their introduction to other Rhizobium species (Lim et al., 1980).

Potential Environmental Benefits and Hazards

Data indicate that a hydrogen uptake system significantly benefits overall plant productivity, since energy supply is a major limiting factor in the fixation of nitrogen by soybeans (Hardy and Havelka, 1975). Hup+ strains of R. japonicum are more efficient symbionts than hup- strains in comparisons of soybean productivity (Lim, 1978; Schubert and Evans, 1977). Also, the plants inoculated with hup+ strains had higher yields than those inoculated with hup- strains (Albrect et al., 1979).

At this time there seems to be no hazard in transferring hup to Rhizobium or other bacterial species that lack these genes since so many bacterial species already possess these genes.

WATER-SPLITTING (lit) GENES FOR GENERATION OF REDUCTANT

The symbiotic fixation of nitrogen is often limited by photosynthetic production by the green plant. Photosynthesis results in the production of both reducing power (NADPH ultimately derived from water) and ATP. The reaction involved is the Hill reaction:

$$\text{light} + H_2O + \text{NADP} \longrightarrow 1/2O_2 + \text{NADPH} + H^+$$

Blue-green algae (cyanobacteria) are the simplest organisms possessing water-splitting genes. Much basic research is required to isolate and characterize these genes before any recombinant DNA techniques can be applied (Andersen et al., 1980). Possibly the water-splitting genes could be transferred from blue-green algae to symbiotic nitrogen-fixing bacteria. The bacteria would still require carbohydrate from the plant, but the bacteria could provide some of its own reducing powder.

DENITRIFICATION (den) GENES

Biological denitrification is the enzymatic conversion of nitrate and nitrite into gaseous forms of nitrogen, including nitrous oxide. Denitrification does not usually occur in well cultivated soils, but it does occur in poorly aerated soils containing an abundant supply of carbohydrates. During the process, oxygen is evolved. A variety of organisms, including Klebsiella and Pseudomonas, contain denitrification (den) genes, but as is the case with the lit genes, much basic research will have to be done before recombinant DNA techniques can be considered. A beneficial aspect of denitrification is that nitrogen pollution can be removed from rivers. Similarly, sewage waters containing high levels of soluble nitrogen can be purified through biological denitrification. One detrimental aspect is that under some circumstances denitrification may contribute to the production of nitrogen oxides (air pollutants found in smog). In addition, denitrification works against nitrogen fixation by diminishing available levels of soil nitrogen and requiring higher inputs of fertilizers (Andersen et al., 1980).

OSMOREGULATORY (osm) GENES

Background

The loss of increasing amounts of agriculturally productive land due to increased salinity is a serious problem worldwide. High salt concentrations in the soil create high osmotic pressures, thereby reducing the availability of soil water to plants and causing plant cells to lose water.

Whole plant mechanisms for combatting excessive salt levels in the environment include osmotic barriers in the roots, swelling of leaves and other structures by absorbance of water which keeps the salt concentration constant and results in succulence, and exudation of salt on plant surfaces or by salt glands. Such mechanisms are under complex genetic control and are not easily amenable to genetic engineering. The study of halophytic plants and bacteria also indicates a cellular mechanism that may overcome excessive osmotic stress from the environment. The halophytic plants, which live and reproduce in or on oceans, seashores, estuaries, deltas, salt marshes, and saline desert soils, can survive by increasing the concentration of dissolved solutes within their cells, either by taking up salts or other solutes from the environment or by synthesizing soluble organic compounds (Epstein et al., 1980). The synthesized compounds include amino acids and their derivatives, such as proline, glycine, glutamate, or γ-aminobutyrate; polyols (compounds with three or more hydroxyl groups), such as glycerol and sorbitol; and derivatives of betaine, a substituted ammonium compound (Marx, 1979; Measures, 1975). Bacteria such as Escherichia, Klebsiella, and Salmonella probably use the same mechanisms to combat salt stress, as do plant cells.

A set of genes called osmoregulatory (osm) genes function in many plant and bacterial cells to control the mechanisms that prevent water loss (Flowers et al., 1977). Workers at the University of California, Davis, have been able to transfer plasmids that encode for salt tolerance and L-proline overproduction in mutant strains of Salmonella to related bacteria. Mutant strains were selected by growing bacteria in the presence of normally toxic concentrations of the L-proline analog, L-azetidine-2-carboxylate. Mutants, that excrete L-proline, dilute and thus antagonize the analog. The mutation conferring L-azetidine-2-carboxylate resistance and salt tolerance is closely linked to the locus for the first enzyme of the L-proline biosynthetic pathway, proB, and it is transferred via an F' factor (a plasmid that is transmissible among the Enterobacteriaceae family). Researchers are still many years away from being able to isolate and fully characterize these genes (Marx, 1979; Andersen et al., 1980). However, advances should come more rapidly than might otherwise be expected since over 60 laboratories around the world are investigating the genetic aspects of plants resistance to damaging salt concentrations.

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Plant and cell culture techniques, without recombinant DNA techniques, can also be used to obtain salt-tolerant plants (Rains, 1979; Croughen et al., 1978). This is discussed further in the section on mutation induction using plant tissue culture.

Potential Environmental Benefits and Hazards

Estimates of present saline soils on a worldwide basis range from 1 billion to 2.3 billion acres (Ponnamperuma, 1977; Massoud, 1974). Approximately one-third of the irrigated lands are now increasingly affected by salinity. The affected land amounts to 41 million acres in the U.S. and 198 million acres worldwide. This loss represents about 0.04 percent of the world's total irrigated land. If it remains constant through the year 2000, about 4.3 million acres (approximately 0.9 percent) of the world's total irrigated land will be out of production. These losses have a greater impact on food production than averaged figures suggest because irrigated land is almost always the most productive land in any region. Even assuming average productivity, 4.3 million acres represents the food supply for more than 5.75 million people (Barney, 1980).

In the U.S., salinity problems are becoming most critical in the heavily irrigated southwest and west (Epstein et al., 1980). Irrigation waters often contain a higher soluble salt concentration than rainwater. Unless drainage is provided to carry off the irrigation waters, the water with its dissolved salts sinks deep into the soil. As the water table rises, the salt is carried back to the surface where it can inhibit plant growth and form a mineral crust. Soil amendments and fertilizers add to the problem. In the San Joaquin Valley of California alone, increasing salinity is costing farmers $32 million/year in reduced crop yields (Marx, 1979). About 400,000 acres of irrigated farmland are affected by high, brackish water tables that pose an increasingly serious threat to productivity. About 1.1 million acres - about 13 percent of the total valley - ultimately will become unproductive unless subsurface drainage systems are installed. The salting problems of the valley have been compared to those that resulted in the collapse of civilizations in Mesopotamia and Egypt's upper Nile. Similar problems are also being observed in California's Imperial Valley (Barney, 1980).

Some nations, such as India and Australia, are largely in arid or semi-arid zones, thus most of their agriculture requires irrigation. As more land is put into production and the demands for water from agricultural, mining, recreational, domestic, and industrial interests increase, the problem of increased soil salinity grows.

Conventional breeding techniques currently are being used to adapt plants to saline conditions. An example of the saline culture research is that which has been performed at the University of California, Davis (Epstein, 1977; Epstein and Norlyn, 1977; Epstein et al., 1980; Rains, 1979). Highlights of the research include the following:

- Thousands of genotypes of barley and wheat have been screened.
- In tomatoes, exotic germplasm has been used to transfer salt tolerance from an economically useless species to a commercial line.

- Successful crop selections and new breeds have been field-tested under saline conditions (Epstein et al., 1980).

In view of this progress and because conventional breeding techniques pose no hazards to the environment, recombinant DNA techniques may not prove especially useful in the development of saline-tolerant crops.

The primary benefit of enhancing the salt tolerance of crops through breeding methods is the increased amount of land that can be brought under cultivation (with all of the accompanying economic and social benefits). Lands that are not in production because the soil is saline--naturally, because salt has accumulated under agricultural practice, or because the only available water is saline--could be cultivated with crop plants possessing increased salt tolerance. Crop production would likely be affected by increasing the amount of land under cultivation rather than increasing productivity as is the case with nitrogen fixation. In fact, in the short term it is expected that yields of salt-tolerant crops grown under excessive salinity will be significantly lower than average world yields. However, small yields are better than no yield at all, something that would occur if currently cultivated lines were exposed to excessively saline conditions (Epstein et al., 1980; Barney, 1980).

Enhanced salt tolerance would also affect cropping patterns. However, economic forces (crop prices) as well as the amount of land under cultivation will continue to be the overriding influence on cropping patterns. Perhaps the secondary benefits of cultivating plants with enchanced salt tolerance include better control of soil erosion, maintenance of water table levels, and reduced flooding in some areas. Further geographic, economic, and social studies are needed to assess these effects (Barney, 1980).

Hazards associated with laboratory transfer of osm genes to plants seem small. However, it does seem remotely possible that salt-tolerant germplasm might be transferred in the field to weeds if crop plants with this new trait normally cross with related weed species (Postgate, 1977). Superior characteristics developed by conventional breeding techniques can and in fact now are being transferred to weed species in this manner. The transfer of traits (introgression) between sorghum and related wild types such as Johnson grass is an example. Not only are new traits transferred by introgression to the wild type, but wild types act as a gene pool for some characters of the cultivated forms. If a new cultivated sorghum is introduced, it will gradually acquire some characters of the former cultivated sorghum via introgression with the permanent wild types (Doggett, 1970). The magnitude of the weed problem is not likely to worsen, however. If the new trait allows an extension of a weed's cultivated range or an increase in vigor, the range or vigor of the wild type weed will change proportionately.

CARBOXYLASE (cfx) GENES

Background

To meet the increasing demands of the world's growing population for food, agricultural yields will have to rise an average of over 2 percent a year over the next 25 years. This will approximately double primary food production according to a study of the National Academy of Sciences (1975). Carbon fixation is extremely important in crop productivity because 95 percent of the dry weight of plants is derived from photosynthesis (Bukovac et al., 1975).

Research on photosynthesis has revealed a number of possible ways to increase crop yields:

- Modification of photorespiration and carboxylation reactions
- Enhancement of photosynthetic electron transport
- Enhancement of translocation of photosynthetic products from leaves to other plant parts and
- Enhancement of transport of carbon dioxide from the atmosphere to the chloroplast (Zelitch, 1979).

The mechanisms and genes involved in these processes have only begun to be elucidated. Also, the genes involved in the above processes are located in both the chloroplasts and the nucleus.

Photorespiration and Carboxylation--

Photorespiration as carbon dioxide evolution differs biochemically from normal respiration in the dark and is specifically associated with the oxidation of compounds produced during photosynthesis. In tests on nine species, photorespiration was found to be 2.2 to 4.8 times as great as regular dark respiration (Decker, 1957). At high irradiance, release of carbon dioxide by this process in many C_3 plants is at least fifty percent of the net carbon dioxide fixed by photosynthesis. Photorespiration is undetectable or operates at only marginal rates in C_4 species. The rate of photorespiration is greatly dependent on the oxygen concentration at normal carbon dioxide levels, and the process is strongly inhibited in leaves when the leaf canopy is enriched with carbon dioxide (Jackson and Volk, 1970).

Some researchers have speculated that millions of years ago when the earth's atmosphere contained more carbon dioxide and less oxygen than now, C_3 plants would have flourished. However, as carbon dioxide was absorbed by plants performing photosynthesis and as oxygen levels increased, these species have been left with the evolutionary burden of photorespiration.

By blocking photorespiration, the net photosynthetic incorportion of carbon dioxide in many species might be increased by at least 50 percent. Although work is still to be done on the biochemistry of the process, evidence suggests that the oxygenase activity of ribulose bisphosphate (RuBP) carboxylase furnishes phosphoglycollate, the initial substrate of photorespiration (Andrews and Lorimer, 1978) (Figure 5). Altering the RuBP carboxylase in such a way that oxidative cleavage of RuBP will be decreased or will not occur may reduce photorespiration. However, it is important to determine whether such an alteration is possible and productive before attempting to engineer the genes of higher plants. Studies on mutationally altered RuBP carboxylases that have a range of catalytic activities in strains of the bacterium, Alcaligenes eutrophus, are now underway (Andersen et al., 1980).

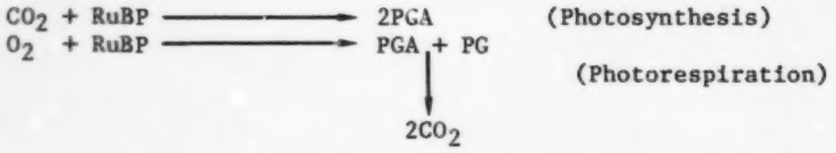

Figure 5. Carboxylase and oxygenase activities of ribulose bisphosphate carboxylase. RuBP = ribulose bisphosphate. PGA = phosphoglyceric acid. PG = phosphoglycollate (Source: Andersen et al., 1980)

A second possible way of increasing carbon dioxide fixation or reducing photorespiration in some species is by transferring C_4 photosynthetic capability to C_3 plants. The biochemistry of the C_4 process is well understood, but the location and function of the genes are not known.

The process involves a complex compartmentation of carbon dioxide-fixing enzymes and carbon transport among cells. The C_4 process is a pump which increases carbon dioxide cencentration in the chloroplast and reduces competition with oxygen (lowers photorespiration). Phosphoenol pyruvate carboxylase functions in the mesophyll cells inside leaves to produce a C_4 acid. This organic acid is then transported to the specialized bundle sheath cells that surround the conducting elements in the leaf, and there the acid is decarboxylated. Finally, RuBP carboxylase fixes the carbon dioxide released in the bundle sheath cells by C_3 reactions of the Calvin cycle (Hatch and Slack, 1970; Laetsch, 1974).

A number of important crop plants (corn, sugarcane, sorghum, foxtail, and finger millets) and forage species (Bermuda grass, Dallis grass, Sudan grass, and Rhodes grass), as well as certain weeds (crabgrass, pigweed, and others), are C_4 plants (Loomis et al., 1971). Leaves of C_4 species have high rates of net carbon dioxide assimilation (42 to 85 mg carbon dioxide per square decimeter per hour) and low rates of photorespiration at high levels of irradiance in air at 25 to 35 C. On the other hand, C_3 species assimilate carbon dioxide at rates about half those of C_4 plants and have rapid rates of photorespiration. The exceptions to this rule, such as sunflower and cattail, are C_3 species with rapid rates of photosynthesis. An understanding of the way these species overcome the rapid photorespiration exhibited by other C_3 plants would provide clues for improving photosynthetic efficiency (Zelitch, 1979).

Transferring the C_4 pathway into some Calvin cycle (C_3) species by conventional breeding of Atriplex rosea (C_4) and Atriplex patula (C_3) yields progeny that have various physiological and biochemical characteristics of photosynthesis intermediate between the parents. Several loci are involved, and transfer is difficult. Other genera known to contain species with both the C_3 Calvin cycle and the C_4 pathway are Kochia, Bassia, Panicum, Euphorbia, and Cyperus. However, interspecific crosses have not been attempted in these genera (Loomis et al., 1971).

Electron Transport--

The rate of photosynthetic electron transport may control carbon dioxide fixation at saturating carbon dioxide levels. This has been shown in experiments with isolated chloroplasts. However, there is no evidence that this is also true for leaves in normal air. Electron transfer between water and $NADP^+$ in the light reactions of photosynthesis takes place along a chain of electron carriers. The carriers are organized into two systems, photosystem I and II, and are connected in series (Velthuys, 1980). The reaction chain of each photosystem contains several hundred chlorophyll molecules, in addition to accessory pigments, lipids, and proteins, that together function as a photosynthetic unit. Light (400 to 700 nm) is collected by chlorophyll molecules, and the energy is transferred to the reaction centers of the photosynthetic unit, where electron transfer begins. Some scientists assume that decreasing the size of the photosynthetic units and increasing the number of reaction centers per chlorophyll molecule would increase photochemical efficiency, but this is yet to be proven. Although researchers are accumulating information on transcription-translation sites of the proteins involved in electron transport (Nasyrov, 1978), much work is still to be done. The genes that control or encode photosynthetic electron transport elements have not been identified or isolated.

Translocation of Photosynthetic Products--

The rate at which photosynthetic products move from leaves to other plant organs may be an internal control of net photosynthesis. It is not currently known whether the rate of translocation is regulated by the loading of carbohydrate into the phloem, by the long distance movement of carbohydrate to its final destination, or by the unloading of carbohydrate at the point of storage or metabolism (Minchin and Troughton, 1980). Accumulation of certain metabolites in leaves may regulate photosynthesis and related processes by feedback-type mechanisms. In addition, translocation may have subtle influences by controlling the concentration of key metabolites that affect photosynthesis. It would be of great importance to be able to control the translocation process, thereby promoting photosynthesis, and also to be able to direct more photosynthate into the part of the plant to be harvested (Zelitch, 1979). However, much physiological research is still required to establish clearly the relationship between photosynthesis and levels of photosynthetic products (Loomis et al., 1971). The genetics of translocation have not been investigated. Applications of genetic engineering techniques are still many years away.

CO_2 Diffusion--

There are several barriers to the diffusion of carbon dioxide from the atmosphere to the chloroplast. These barriers include the diffusion resistances imposed by the microscopic pores (stomata) on the leaf surface, the physical diffusion resistances caused by the aqueous medium of plant cells, and the diffusion resistances caused by carbon dioxide released by respiration and photorespiration. These barriers seem to be much more limiting to photosynthetic rates than photochemistry or enzymatic carboxylation. The effects of diffusion barriers may be reduced by optimizing the interception of solar radiation, by planting varieties whose leaves expand rapidly, by spacing plants to improve light interception, and by increasing the ratio of growing to senescent leaves (Loomis et al., 1979; Zelitch, 1971). Much physiological research is still required in this area, and applications of recombinant DNA techniques seem remote at this time.

Potential Environmental Benefits and Hazards

Since the recombinant DNA technology needed to improve the photosynthetic efficiency of crop plants may require years to develop, it is difficult to analyze its impact thoroughly. The benefits of higher productivity of wheat and other crop plants would be profound. For example, higher yields per unit of land area could be achieved without increasing the amount of fertilizer or pesticide used. This could result in better nutrition in less developed countries and lower prices with increased export trade in the developed countries.

A potential hazard, mentioned in the sections on nitrogen fixation and salt tolerance, is the transfer of enhanced photosynthetic qualities to weed species, a situation that might eventually require a greater use of herbicides.

INDUCTION OF MUTATIONS BY PLANT TISSUE CULTURE TECHNIQUES

Background

Such plant cell and tissue culture techniques as the selection of somatic-cell variants, cell fusion, and the induction and selection of mutants, can create genetic variability and modify the genetics of plants, without resort to recombinant DNA techniques. Plant tissue culture techniques have been used to select cell lines tolerant to salinity, herbicides, and disease. Among the cell lines selected are:

- Tobacco and bell pepper lines tolerant to salinities as high as 1 to 2 percent (Dix and Street, 1975).
- Wheat (Rains, 1979) and alfalfa (Croughen et al., 1978) with enhanced salt tolerance.
- Tobacco (Chaleff and Parsons, 1978; Radin and Carlson, 1978) and white clover (Oswald et al., 1977) lines selected for herbicide tolerance.
- Sugarcane lines resistant to *Helminthosporium sacchari* sugarcane mosaic virus, and *Sclerosporia sacchari* (Nickell, 1977).

- Potato lines with increased frequency of genetic variation for agronomically valuable characteristics such as disease resistance (Shepard et al., 1980; Gwynne, 1980).

- Texas male-sterile cytoplasm maize lines resistant to *Helminthosporium maydis* race T pathotoxin (Gengenbach et al., 1977).

However, while selection for cell lines tolerant to stress conditions has been accomplished, testing for genetic stability and tolerance at the whole plant level has rarely been attempted.

The above-mentioned species are all vegetable or grain crops. Tissue culture techniques can also be used to modify other plant species and to shorten the time required to develop them as new fuel or fiber crops. Many plants, which are commonly considered weeds because they compete with crops or other desirable plants, are now being considered as potential crops themselves (Buchanan et al., 1978; Saterson et al., 1979). Among such non-conventional, weed crops are:

- Common milkweed for fats, proteins, hydrocarbons, rubber, and bast fibers.

- Giant ragweed for proteins, oils, rubber, paper pulp, and biomass for fuel production.

- Jimsonweed for alkaloids as well as for biomass for fuel production.

- *Euphorbia* (various species) for hydrocarbons and epoxy fatty acids.

So far, applications of tissue culture to new crop development have been confined primarily to model systems. For example, tissue culture techniques have been applied to milkweed (Groet and Kidd, 1980) as well as to *Euphorbia* (Biesboer and Mahlberg, 1979). However, large-scale propagation for field planting or selection of stress-tolerant strains has not yet been attempted.

Potential Environmental Benefits and Hazards

The environmental benefits and hazards of tissue culture technology are not expected to be different from those due to the development of new varieties of crops by conventional breeding alone. Utilization of tissue culture would merely shorten the time needed for development.

Development of new crops from weed species is desirable for numerous reasons (Princen, 1977):

- To reduce dependency on foreign imports for a variety of raw materials, such as natural rubber and vegetable oils.

- To reduce shortages of specialty chemicals, such as essential oils, tannins, and alkaloids, and other materials, such as waxes, fibers, fats, and oils.

- To provide new protein sources for livestock feed in areas where established crops cannot grow.

- To increase land utilization by developing species adapted to marginal conditions.

- To develop new specialty crops for small farm regions throughout the world that are not amenable to monoculture.

Increased competition with conventional crops is likely to be the major environmental concern for most weed or nuisance species considered for crop development. As a result, more herbicides would have to be used, and new selective herbicides would have to be developed. In previously undeveloped areas, competition between superior varieties of weed crops and the native flora could produce changes in species composition and succession, and ultimately, a reduction in the stability of the ecosystem. This may be particularly true in fragile ecosystems, such as desert, alpine, and coastal regions. However, the likelihood that there will be increased competition seems very slim especially since modifications of the weed species for use in conventional agricultural systems will probably render them less competitive.

SUMMARY

Tables 6 and 7 list the potential environmental benefits and hazards that might be derived by applying genetic engineering technology to agricultural problems. The benefits and hazards are roughly listed in their order of importance with the most important being listed first. Secondary effects are listed towards the bottom of the list.

A SCENARIO ON THE IMPACT OF GENETIC ENGINEERING ON AGRICULTURE 1990--A GLOBAL PERSPECTIVE*

Political Situation

The United States (U.S.) and, to a lesser degree, other Organization for Economic Cooperation and Development (OECD) countries have drifted toward policies engendered by a conservative political climate. Confronted with progressively more aggressive global strategies by the U.S.S.R. and other Communist-Bloc countries, and having fully overcome the post-Vietnam psychology, the U.S. has consistently employed a policy of containment and peace-through-strength in its dealings with the Soviet Union and its allies. Backed by conservative factions, the U.S. and its European partners during the 1980s have been successful in increasing military expenditures in their national budgets by 50 percent, including defense-related R&D expenditures.

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* The reader is reminded that the author of the scenario is in the year 1990 looking back over the 1980's and also speculating about events to the year 2000.

TABLE 6. POTENTIAL ENVIRONMENTAL BENEFITS OF AGRICULTURAL GENETIC ENGINEERING

Decrease nitrogen fertilizer input by conferring nitrogen-fixing ability on nonlegumes or by increasing the nitrogen-assimilating ability of nitrogen fixing microbes

Increase the amount of land under cultivation and increase marginal land usage

Increase tolerance of plants to environmental stresses (e.g., salts, pests, drought, frost, pollutants)

Enhance development of underexploited crops for multiple end uses (feed, fuel, fiber)

Improve yields in agriculture and silviculture by hastening cultivar selection and development

Increase crop uniformity and decrease culling of grains and vegetables

Improve nutritional value of seeds, fruits, and vegetables

Decrease cost of crop production

Decrease dependence on foreign supplies of oil and make farming less energy intensive

Enhance competitiveness of pioneer plant and microbial species (revegetation)

Decrease pollution and eutrophication of aquatic environments caused by pesticide and fertilizer run-off

Create new agricultural products (e.g., encapsulated pesticide-degrading microbes)

Decrease use of chemical pesticides by construction of effective microbial insecticides

Expand basic knowledge of gene structure, function, and regulation and the molecular mechanisms that control plant productivity

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TABLE 7. POTENTIAL ENVIRONMENTAL HAZARDS OF AGRICULTURAL GENETIC ENGINEERING

Produce more vigorous weeds, and thus increase development and use of herbicides
Alter the niches and pathogenicities of plant viruses and soil bacteria
Increase breakdown of fertilizer and increase production of nitrogen oxides (denitrification)
Increase rate of organic matter decomposition (e.g., by _Erwinia_) in the soil
Decrease the range and variability of some crops, thereby inadvertently increasing disease susceptibility

Polarization of the world political situation, leading to the clear identification of hostile as well as friendly nations, has markedly reduced the anxiety and uncertainty that plagued the west in the late 1970s and early 1980s.

Social-Economic Situation

Population--

The world population has increased at an annual rate just under 2 percent since 1980, and that growth rate is expected to continue through the year 2000. There are now about 5 billion people in the world, and that number is expected to swell to about 6 billion over the next decade. The population of the U.S. is now 245 million people. Over 90 percent of the world's population growth has occurred in the less developed countries (LDCs). This means that of the 5 billion people now in the world, over 3.8 billion live in LDCs. Currently, the average life expectancy is 55 years. Unlike the 1970s, the increase during the 1980s was due more to improved health care than to increased availability of food (see Food/Agriculture). However, in certain countries (e.g., India), food production was the overriding factor. Over the past ten years, fertility has dropped by an average of 25 percent, from an average of four children per woman to three.

In addition to rapid population growth, the LDCs have experienced extreme alterations in population distribution during the 1980s. Dramatic movement of people from the countryside to the city continued. Many cities in LDCs became remarkably large and overcrowded. For instance, greater Mexico City, Sao Paulo, and Seoul are much larger than New York City (now about 12 million people). The bulk of the people have migrated to the cities still live in slums or shantytowns where education, sanitation, and other public services are minimal or nonexistent. The difficulty of maintaining conditions during uncontrolled urban growth occurred alongside worsening conditions in the rural areas of the LDCs. Lack of job opportunities has continued to be severe in these outlying areas, forcing increased migration to the cities.

The large urban populations have resulted in an extreme need for maximum agricultural production in the rural areas. The low income of the average family, however, has created a gap between the felt need for increased agricultural production and the economic demand for this production. Agricultural production even decreased in some countries because governments placed artificial ceilings on food prices in response to urban political pressure. This left farmers unable to recover costs if they were to employ the high energy inputs necessary for maximum production and for expansion to unused land.

Economics--

Because population grew rapidly, the worldwide average GNP now stands at about $2400 per capita compared to $1500 in 1975. The rate of growth in worldwide GNP began declining in 1985.

The GNP growth rate in the U.S. and other OECD countries for the past ten years has ranged from 2.5 to 3.0 percent annually, so that the per capita

GNP now averages more than $9500. The inflation-recession cycles are still evident, but through the 1980s OECD countries maintained better control than they did during the 1970s. The U.S. inflation rate was slightly over 8 percent last year (i.e., 1989), and its GNP grew 2.7 percent.

By contrast, the LDCs have witnessed economies expanding at near 6 percent annually over the past decade. Per capita GNP in the LDCs, now averages less than $450 while these indicators point to some progress in economic stability in the LDCs. The economic disparities between wealthy and poor nations continue.

Energy--

The energy picture has become increasingly gloomy. Over the last decade there were large increases in demand for all commercial energy resources. The world energy demand is currently 390 quads. Oil is still the world's leading energy source, providing 45 percent of the world's energy supply. The average price for a barrel of crude oil has reached $65.00 (1980 dollars). In view of the increases in demand, less than adequate successes in conservation, and development of alternative energy sources by the western countries, the $65.00 per barrel seems considerably low. Two reasons can be cited for this relatively moderate price. First, during early parts of the 1980s the moderate elements within OPEC were successful in consistently dissuading the more radical OPEC countries from raising oil prices in the manner of 1970s. The winning argument has been the fear of economic collapse in the western countries and its consequences for the oil-producing nations. Second, a succession of inter and intra political upheavals within and between oil-producing countries in the middle-East contributed to disunity within OPEC ranks, thus preventing OPEC from acting in unison on the oil price issue.

Although the price of oil is considered manageable by the western economies the oil-producing countries in the middle-East have proved to be unreliable sources of supply. Constant interruption of supply due to internal political turmoils and between countries squabbles have at times thrown the western world into shocks reminiscent of the oil shortages in the 1970s.

The per capita consumption of energy has increased about 70 percent in industrialized countries other than the U.S. The U.S. and LDCs both witnessed a 30 percent increase in energy consumption over the 1980s. In actual values annual per capita consumption of energy is about 400 million Btu in the U.S. compared to about 15 million Btu in developing nations.

The short supply of wood for fuel has created a serious problem for the LDCs. Over the 1980s the demand for wood increased 25 percent, but the availability of wood decreased about 75 percent. There are virtually no forests left in sub-Saharan and central Africa, in southern Asia, and in certain Caribbean and Central American countries. The poor burn only crop residues and dung. Extensive cutting of forests in Southeast Asia and other Asian and African countries has also destroyed water supplies by altering forested mountain watersheds.

Food/Agriculture--

Over the decade of the 1980s, food and fuel needs around the world became the most pressing human problems.

Through the 1980s, world food production increased at an average rate of slightly over 2 percent per year, a rate of increase is expected to continue through the year 2000. So, estimates indicate that by 2000 food production will be 80 percent greater than it was in the base year 1970.

Maintenance of these high yields depends on the continued use of energy-intensive inputs like nitrogen fertilizer, pesticides, herbicides, and irrigation. Energy-intensive production methods continued and will continue to aggravate further fuel availability and oil prices. Intensive agricultural practices and the use of more marginal land have greatly enhanced desertification. Thirty million acres of arable lands were lost during the 1980s. Most good land is already under cultivation, and from 1990 to 2000 land under productive cultivation will increase only about 4 percent (mostly due to the annexation of marginal, sloping lands and to the clearing of forested land). So, by 2000, 1 acre of land should supply food for 10 people, whereas in 1970 1 acre fed about 6 people.

During the 1980s world food production barely kept ahead of the world population; the absolute price of food increased 55 percent. This increase was a direct result of increased petroleum dependence and the shunting of food/feed reserves (corn grain) to the manufacture of liquid fuels, although by the late 1980s grain-based alcohol production was supplanted by coal-to-methanol processes. In the U.S., the cost of producing crops increased about 12 percent per year through the 1980s. Production-cost increases in the LDCs were somewhat lower but still fiscally burdensome to those countries. Adding to the rapidly increasing food production costs was the doubling of food distribution costs (also petroleum dependent) during the decade.

Per capita food consumption in industrialized nations has increased on the average by 15 percent since 1980 and is projected to increase another 15 percent by 2000. Over the past 10 years, per capita food consumption increased about 20 percent in Japan, Eastern Europe, and the U.S.S.R., and about 10 percent in the U.S. and the LDCs. This relatively small overall increase in consumption in the LDCs in misleading because there were enormous variations in consumption among nations and regions. Similar consumption increases in the U.S. and the LDCs have occurred for different reasons. For example, the U.S. experienced an average population growth (about 1 to 2 percent of world growth) while the LDCs saw rapidly increasing populations but relatively low agricultural productivity. The number of malnourished people in the LDCs now stands at almost 2 billion.

Technological Situation

Global R&D Expenditures--

The total R&D expenditure is still greater in the U.S. than in any other OECD country. However, the ratio of R&D expenditures to GNP in the U.S. has declined consistently since the 1960s and stood at 2.0 percent by the middle of the 1980s. At the same time, the R&D to GNP ratio for West Germany and

Japan has surpassed that of the U.S. Throughout the 1970s and early 1980s the general pattern within the OECD countries, except for the U.S., was to shift away from military to civilian R&D. Recently there has been an observable and uniform reversal of this trend with almost all OECD governments now expending larger portions of their R&D funds for military-related applications. The nonmilitary research areas that have received special attention in recent years in the U.S. are energy development and conversion, human health, and the environment.

The level of R&D expenditure by industry in the individual OECD countries varied with the performance of the respective economies. Japan and West Germany have enjoyed healthy and growing economies and consequent steady growth in basic, applied, and developmental R&D expenditures by their industries. Even though the economic growth has been only moderate and the inflationary-recessionary cycles have abated in the U.S., industry has become more selective and cautious in its R&D allocations. Expenditure for basic and applied research by U.S. industry still lags behind the U.S. Government's. However, several technologies in the OECD countries have benefited from exceptionally high industrial investment in both basic and applied research activities. Energy-related technologies, chemical and allied technologies, drugs and medicines, food technologies, and electrical and communication technologies are among the prime examples.

Genetic Engineering in General--

Against the foregoing political and economic background, genetic engineering had some success in the 1980s, but fell short of the early promises that attributed broad applications of panacean proportions to the technology.

The expectations in the early 1980s about the potential of genetic engineering propelled a large number of high technology industries, such as the chemical industry, into investing in basic and applied research in genetics. Genetic engineering research programs were also begun by several academic and nonprofit research institutions in the U.S. and several other OECD countries, such as Germany and France. Some countries, such as Japan, which by the early 1980s already had in place an infrastructure for developmental research in fermentation aimed at specific medicinal and fuel applications, broadened their efforts toward many end-uses. This effort was reinfored by the increased exchange of scientific and technological information and products among the OECD countries. OECD countries have slowly moved toward a course of "less protectionism" in trade, science, and technology among themselves, in part as a countervailing policy against actions of the U.S.S.R.

This increasing international cooperation among the OECD countries created employment and economic opportunities for many biological scientists. Up through the first quarter of the decade, the opportunities were mostly reserved for physical scientists, and more specifically for those who were active in research on energy engineering, medicine, and national defense. Industrial organizations with in-house R&D activities in genetic engineering, profit-making and nonprofit R&D institutions, and organizations specializing in genetic engineering created a highly competitive employment market for scientists with advanced degrees in the biological sciences. The demand for specialized scientists in genetic engineering was further heightened by the short supply which resulted from the overcommitment of resources in the late 1970s and early 1980s to postgraduate programs other than life sciences. For example, the concern over the inadequate supply of scientists and engineers in such energy-related fields as mining, petroleum engineering and chemical engineering attracted considerable resources in the U.S. Although most institutions of higher learning in the OECD countries had made the adjustment by the mid-1980s and more doctoral-level biological scientists were entering the market, most of the pioneering research was still being carried out in a few organizations and by experienced scientists whose number was less than the growing demand for their know-how.

In the U.S., federal and industrial R&D funding for genetic engineering has grown to $220 and $500 million annually, respectively. Over 180 industrial organizations are now performing genetic engineering R&D. The recombinant DNA products market alone has grown to $3 billion.

Because of the inadequate supply of highly trained scientists needed for the pioneering work in genetic engineering and because of unforeseen experimental problems, obtaining results from the work carried out in the development and application phases has become inhibitively prolonged and costly. For instance, interferon production via recombinant bacteria was greatly slowed in the mid-1980s because of successful production from animal cells. Also, human insulin and growth hormone production from bacteria just received full approval from FDA in 1986.

During the 1980s, applications of genetic engineering technology encountered stiff competition from newly developed organic chemical technology, new drug formulation and delivery technologies, and a resurgent interest in conventional plant and animal breeding. Even though genetic engineering has made moderate progress overall, replacement of traditional industrial processes (e.g., the Haber-Bosch process for producing ammonium fertilizers) are not being considered.

Agriculture and Genetic Engineering--

During the 1980s, crop improvement programs centered on overcoming problems arising from the increased pressures put on soils throughout the world. In both industrialized countries and LDCs there has been a spread of desert-like conditions in the drier regions and heavy erosion in the more humid regions. The increased destruction of vegetation cover, the loss in yield, and the decrease in the availability and fertility of soil have mainly been caused by overgrazing, destructive cropping practices, urban encroachment, and use of plants for fuel.

The impetus for the development of genetic engineering in agriculture over the 1980s rose primarily from destructive cropping practices such as irrigation and pesticide application. The loss of irrigated lands over the decades has been significant. Today (1990) about 18 percent of the world's arable 125 million acres is being irrigated. Productivity on about 60 percent of the world's irrigated land has decreased because of increased salinity, alkalinity, and waterlogging. Irrigation's negative impacts on productivity exist in both industrialized countries and LDCs. The extensive use of pesticides into the late 1980s, which sustained the rise in crop yields, resulted in increased resistance of most pests to multiple pesticides and in the destruction of pest-predator populations.

Even with the impact of the major seed companies and oil companies entering plant genetic engineering in the early 1980s, the application of genetic engineering to agriculture has fallen short of expectations. In the early 1980s proponents of short-term benefits to agriculture minimized the problem of techniques still to be developed. Even now, no commercially available seed stock contains a new crop gene introduced by genetic engineering. Corn with the capacity to fix nitrogen is closest to commercialization, another six years being required to test the stability of the trait and to produce hybrid seed in quantity. Delays in producing nitrogen-fixing corn have arisen because most strains that fix nitrogen did not retain other attributes judged essential by farmers (e.g., high yield, pest resistance). Other plants, mainly vegetable crops, have been engineered to be salt-tolerant, herbicide-resistant, etc., but commercial seeds with those traits are not yet available.

In the early 1980s, the possibility of growing isolated plant cells in culture and the subsequent regeneration of whole, seed-bearing, competent plants offered promise in breeding new crop varieties. For instance, during the 1980s cell lines of corn and wheat resistant to specific toxins from microbial pathogens were developed, but the whole plants derived from these cell lines possessed poor grain quality. In any event, conventional wheat and corn breeding have already provided the world with many strains resistant to toxin-producing pathogens.

Other factors during the 1980s limited the application of genetic engineering to agriculture. First, funding for basic research in the plant sciences decreased despite world food pressures, because of emphasis on military research. Our data base for scientific experimentation with photosynthetic plants is still more meager than that for health and human research. Second, concern over the impact of using recombinant organisms (including plants) in the environment slowed research in the early 1980s. Third, genetic engineering was aimed at increasing the uniformity of crops. Unfortunately, large monocultures of genetically identical crops increase the risk of catastrophic loss from insect attacks or crop epidemics. The corn blight that struck the U.S. corn belt in 1970 was a clear illustration of the vulnerability of genetically identical monocultures. The possibility of other such epidemics was constantly being raised during the 1980s, thereby impeding research on genetic engineering of plants.

The 1980s further confirmed the idea that genetic engineering must function only as an adjunct to, rather than as a replacement for, conventional plant breeding. Through the 1980s conventional plant breeding has been successful and safe to the environment. Even today the limits to improvement of the major grain crops through conventional plant breeding have not yet been attained. For example, in 1930 average U.S. corn yield was 20 bushels/acre (bu/a), in 1975 86 bu/a, and in 1990 105 bu/a. In another instance, wheat productivity was 14 bu/a in 1930, 30 bu/a in 1975, and

38 bu/a in 1990. The productivity of corn and wheat are still increasing, although at an ever slower rate. The increased yields were due to conventional breeding of superior stock as well as to increased use of energy-intensive factors (fertilizers, pesticides, harvesting equipment).

In addition to the research thrusts in plant improvement that have been on-going for several years, new problems are beginning to spawn new areas of investigation. The versatility, proven record of success, comparatively low technology needs, and consequently moderage cost of conventional plant breeding have resulted in the lead role for both the old and the new research efforts being in the hands of plant breeders. Genetic engineering technology has become, and will continue to be, a tool of last resort for increasing the genetic diversity of a species. It is used by the breeders to provide the occasional important breakthrough they could not otherwise expect.

The steadily worsening impact of chemicals on the environment has resulted in several major research thrusts in plant improvement. Two approaches are being taken to deal with soil contamination with heavy metals, such as sewage sludge, with certain landfill areas, and with reclaimed areas formed from dredgings from rivers and lakes. A variety of plants that take up heavy metals from the soil are being developed for growing on contaminated soil to decrease its metal content. Some are grain-producing crops which do not translocate metals to the grain. The grain will then be fed to animals, and the remaining biomass combusted for energy with the ashes going to hazardous waste areas. Other plants that do not take up specific metals are being developed so that metal-free crops can be grown on contaminated land but used freely in normal commerce. The need for plants resistant to air pollutants is increasing, both because larger geographical areas are affected by pollutants and because higher maximum concentrations are now encountered in certain localities. Certain especially sensitive crops are being developed for increased resistance to ultraviolet radiation in anticipation of continued depletion of the ozone barrier in the atmosphere.

The energy crisis has crippled the economies of many LDCs so that the use of fertilizers and disease-control chemicals has considerbly diminished. The resulting impact is heightened because in most tropical settings there are greater pressures from pests and faster leaching of nutrients. One new thrust that is arising in response to this situation is designed to make increased use of mycorrhizae-plant associations. Although such associations are common to most plants, not all mycorrhizae are equally efficient in increasing nutrient availability in poor soils. Genetic engineering techniques are being used to attempt the transfer of appropriate genes from species hosting the most beneficial mycorrhizae to other plants to enable them to form similar symbiotic associations. Attempts are being made to transfer genes coding for rotenone, pyrethrum, and other natural insecticides to a variety of plants to give them a natural resistance to insects. Genes responsible for the ability of certain plants to secrete phytotoxic substances from the roots and for resistance of the host plant to those substances may soon be transferred to create herbicide-producing crops. The potential of these developments for reducing energy requirements is beginning to attract considerable attention.

The depletion of forests in nations where 90 percent of the families rely on charcoal as fuel for cooking has resulted in an extreme crisis, with charcoal as well as food now being sent by relief agencies. Research is now on a crash basis to develop varieties of local staples that require less cooking. The pivotal role of pulses as the major protein source in many countries has resulted in special attention being given to them. Efforts to reduce their exceptionally long cooking times is still at the basic research level. Equally important are efforts to develop varieties that lack the antinutritional and toxic substances that require long cooking times to inactivate. Countless edible plants that have scarcely been utilized until now are being screened as potential new food sources that can be consumed raw or that can be prepared with minimal cooking.

Regulation of Genetic Engineering and its Impact on Agriculture--

The regulations concerning genetic engineering applications in agriculture during the 1980s fall into five classes. These are:

- Research practices and procedures
- Types and species of plants
- Types of micro-organisms
- Industrial scale-up and field testing
- Proprietary issues, e.g., Plant Protection Act

The areas of agriculture that have been impacted due to the enforcement of the regulations are:

- Investment
- Industrial R&D expenditure
- Basic and applied research and technical developments

Two sectors have been concerned with compliance with these regulations.

- Academic sector
- Industrial sector

The investment inertia, that was created in the early 1980s due to expectations for high long-term returns, has been sustained throughout the decade. However, there have been short periods when the investors reacted cautiously to industrial organizations whose programs of R&D in genetic engineering were highly susceptible to regulatory pressures. When an industrial organization's R&D programs required reconsideration by existing regulations, generation of capital became a major problem. The investors simply would not commit their money until the regulatory issues were resolved.

Among the five areas of regulation, none has had more impact upon investment than the regulations related to the industrial scale-up and field testing. As the last stage in events prior to full commercialization, scale-up and field testing represent the point at which most of the developmental expenditures are considered sunk cost. Unless products or processes are forthcoming, the incurred costs cannot be recovered, and profit remains unrealized. From the investors point of view, delays due to regulatory considerations mean increased risk of return on capital. For the organization, it means a tie-up of expenditure and the slow down of cash flow and profit. Consequently, when compliance with regulation aimed at scale-up and field testing was involved, investors and companies were discouraged from committing themselves, or they acted cautiously in commitment of capital and organizational effort.

Compliance with regulations in the area of research practices and procedures has had different degrees of impact upon investment in R&D. In industry, the enforcement of research procedures and related regulations has progressively resulted in greater R&D costs. The increase in cost has been most noticeable for the experiments requiring P3 and P4 containment laboratories. These regulations have meant more elaborate and sophisticated laboratory construction. Capital-rich industrial organizations have been able to proceed with their R&D. However, smaller organizations engaged in recombinant DNA research have been forced to limit their investigations as dictated by their containment capabilities. Universities, which are traditionally bounded by the availability of funds, have been impacted more severely. A number of universities have been forced to abandon some of their genetic engineering research activities since 1985. On the other hand, academic institutions with historically strong financial ties have successfully complied with the containment requirements. As a consequence, a handful of universities have attracted most of the scientists working in agricultural genetic engineering.

The strict enforcement of research practice regulations have increased the direct labor cost of compliance both in the industrial and academic environments. For example, in most of the universities clerical staff now handle the paperwork and communications related to the regulatory functions. Compliance requirements have also forced the bench researchers at the universities to spend more time not directly related to experimentation. Although this factor has not actually prolonged the research time, it has become a source of complaint and frustration for academic scientists. In industry, increase in the scale of organizational structure has been partly due to regulations in research practice. Industry, unlike universities, has successfully shielded its scientific staff from devoting time to compliance matters. This has been done by increasing the number of organizational levels and specialized personnel.

Regulations on species of plants and micro-organisms have dramatically impacted basic and applied research. The effects of these regulations have produced two major consequences. The first, and most obvious, has been limitations upon the scope of research, thus limiting applications of new plants and processes. This effect has been most severe on species of crop plants with large commercial potential. Second, many in the scientific community contended throughout the 1980s that regulation of species must be based upon data that, they claim, do not yet exist. Industry has reacted to these regulations by simply shifting its R&D emphasis to the unregulated areas, without any noticeable loss of enthusiasm for potential commercial payoffs or without reduction of expenditure. Investment has been directed towards commercial ventures whose underlying R&D programs and production processes utilize unregulated plant species and micro-organisms.

A significant event in the 1980s has been the development of relatively undisputable and clear rules for identifying the characteristics of genetically engineered plants to be patented. This has given added impetus and incentive to industrial concerns. The perceived commercial potentials have frequently accelerated investment in companies whose proprietary potential for new plants seemed attractive. Although the impact of genetic engineering in development of new plants has been less than anticipated and only a limited number of patents issued, the financial community has acted more on expectations than on actual results.

An argument frequently heard in the early 1980s was that patents may restrain the free exchange of scientific information. This contention has neither been proved or refuted by the experiences of the last few years. However, as a result of patent issues, the scientists are now more inclined to discuss their techniques because the threat of duplication of process are protected by law.

In summary, the regulatory impacts in the 1980s have ranged from negligible to moderate. The regulations have not dampened investment, generally. They have increased the cost of R&D but not to a prohibitive degree (at least for industry). The academic sector, however, has not been able to adapt as easily as industry. Industry has pushed ahead within the regulatory framework. On the other hand, the university scientists, who historically carry out the basic research, have produced less results both in terms of scale and scope.

SECTION 3

RECOMMENDATIONS ON REGULATION AND TECHNICAL INFORMATION NEEDS

Recombinant DNA technology can be an excellent tool for changing the genetic complement of plants. The wealth of information about bacterial genetics contrasts sharply with the paucity of data concerning the genetics of complex animals and plants. Therefore, a great deal of basic technical information still needs to be obtained before the gaps in the information required for determining regulatory actions will be apparent. Many previously identified gaps, such as how to label and track genes, special training of personnel involved in genetic engineering laboratory work, emergency clean-up procedures, and techniques for preparing environmental impact statements appropriate for recombinant DNA technology, are already being addressed by NIH, EPA, and other agencies. The unique research gaps identified during this project are preventative weed research, new pheromone research, and vector control.

- Preventative weed research.

Introduction of plant germplasm into the U.S. from abroad has, unfortunately, produced some highly undesirable weeds. Introduction of engineered plants with new desirable traits or introduction of existing plants (weeds) as new crops could have a similar negative result. Preventative weed research on each engineered species should be done. This would involve literature studies and possibly small-scale field testing. Plants should be examined for (1) capacity to invade waterways, croplands, grazing lands, and forests; (2) methods of dissemination and longevity of seed or other reproductive structures; and (3) other undesirable characteristics such as the presence of toxic substances associated with the genera or family to which the species belongs. Suspect plants discovered in the literature study should be field tested under carefully controlled conditions so that no seeds, rhizomes, or other reproductive materials escape. The plants should be subject to quick eradication if needed. Simple competition studies could be conducted by planting the species under examination in cultivated fields. Plants suspected of containing toxic substances could be treated in bioassays in which dried plants, extracts, or residues are fed to experimental animals (Williams, 1980). If such studies had been conducted prior to introduction of kudzu vine (Pueraria lobata) or Hydrilla (Hydrilla verticillata), these possibly would not be major pests in the U.S. today. These plants were introduced for erosion control and for use in aquaria, respectively. In general, the probability of engineered species becoming pests would probably not be as great as that of foreign species introduced for breeding or economic purposes.

- Chemical Interactions Between Species.

The intentional or unintentional introduction of genes into plants for production of various toxic chemicals could have ecological effects. Such genes would be intentionally introduced for the purpose of providing crop plants with protection against various pests. Many secondary substances produced by plants (presumably evolved as repellents) are used by man for flavors, scents, and drugs. The alkaloids, caffeine and nicotine, are among these. In addition, some plants produce natural insecticides, such as pyrethrum or rotenone. Changes in the amounts or types of these compounds in plants could effect the adaptation of species and the organization of communities. Chemical messages (pheromones) between members of a species are signals for reproductive behavior, social regulation, control of caste differentiation, alarm and defense, territory and trail marking, and food location. Other chemicals (allelochemicals) produced by organisms of one species affect the growth, health, behavior, or population biology of other species (Whittaker and Feeny, 1971).

Research involving literature studies and possibly small-scale field testing or soil microcosm studies should be conducted on engineered species, particularly those involving transfer of genes for chemical production. Testing would be very similar to that suggested for preventative weed research. Highly controlled field or microcosm tests could be designed according to the species and ecosystems involved. As was mentioned under preventative weed research, the probability of engineered species causing changes in species adaptation and community organization would probably not be as great as that of introduced foreign species or as that of a pollution-producing industry.

- Vector Control.

Several features of *Agrobacterium* could promote the spread of pTi DNA throughout the environment. The Ti plasmids are conjugative in that they carry genes that enhance conjugation and transfer of Ti-plasmids between donor and acceptor bacteria (Kerr et al., 1977). The host range for Ti plasmids has been further extended by production of hybrid plasmids from the Ti plasmids and the wide-ranging plasmid RP4. The Ti-RP4 plasmid can be transferred by conjugation at a high frequency to many other gram-negative bacteria, such as *Pseudomonas*, *Rhizobium*, *Escherichia*, and *Erwinia* (Schell and Montagu, 1978) and to other *Agrobacterium* species. For instance, plasmid transfer may produce more virulent strains of crown-gall, hairy root (White and Nester, 1980) and other plant diseases.

The caulimoviruses display several biological characteristics that could make them relatively safe genetic vectors. They have a restricted host range and will not infect important grain crops such as corn or wheat. Mechanical transmission is difficult, and the virus is not transmitted by seed or pollen. Geminiviruses, on the other hand, have a wide host range, including grain crops and legumes. In addition, accidental infection of the natural insect vectors could be disastrous, since the insects maintain the ability to infect plants throughout their lifetime. If geminiviruses are to be seriously considered as vectors in genetic engineering experiments, attention should be given to developing noninsect-transmitted strains, such as have been developed for the caulimoviruses. As with the caulimoviruses, mechanical transmission of geminiviruses to plants is difficult, and the virus is not transmissible by seed or pollen.

The possiblity that manipulations involving either Agrobacterium or plant viruses could provide these organisms with increased pathogenicity seems remote, and the possibility that such an organism would be accidentally released to the environment seems even less remote. However, if such an organism was released accidentally into the environment, it might be more competitive than the nonmanipulated organisms. In addition, transfer of genes to other bacteria by conjugation could produce other, possibly, pathogenic strains.

For these reasons, containment requirements for experiments involving Agrobacterium and plant viruses should be evaluated periodically. Highly controlled experiments involving transfer of nif or osm genes with such vectors should be attempted to determine how much uncontrolled transfer of the genes might occur in nature.

Finally, EPA should continue to solicit advice and recommendations from scientists working in the field of DNA recombination. Since so much is still being learned about this new technology, policies must be set with the expectation that they may later be reversed instead of requiring continuous compliance.

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APPENDIX A

A PARTIAL LIST OF SCIENTIFIC CONFERENCES ON PLANT GENETIC ENGINEERING

APPENDIX A

TABLE A-1. A PARTIAL LIST OF SCIENTIFIC CONFERENCES ON PLANT GENETIC ENGINEERING

Dates	Conference	Location	Organizer
November 30 - December 5, 1980	Joint meeting of American Society of Agronomy Crop Science Society of America Soil Science Society of America	Detroit, Michigan	Societies Business Office 677 South Segoe Road Madison, Wisconsin 53711
December 1-5, 1980	Fourth International Symposium on Nitrogen Fixation	Canberra, Australia	A.H. Gibson Division of Plant Industry C.S.I.R.O. Box 1600 Canberra City 2601 Australia
December 19, 1980	Genetic Engineering	Chelsea College London, England	Society for Drug Research
1980 (exact dates not available)	IX General Congress European Association for Research on Plant Breeding	USSR/(city unknown)	
January 26-30, 1981	Gordon Conference on Agriculture	Ventura, California	Dr. Richard J. Patterson University of North Carolina Greensboro, North Carolina 27410
February 25-27, 1981	First International Congress for Recombinant DNA Research	San Francisco, California	Edward Ruffing Congress for Recombinant DNA Research c/o Scherago Associates, Inc. 1515 Broadway New York, New York 10036 (212) 730-1050

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
March 1-6, 1981	American Society for Microbiology	Dallas, Texas	Robert Watkins American Society for Microbiology 1913 I Street, N.W. Washington, D.C. 20006
March 9-13, 1981	International Symposium on Induced Mutations as a Tool for Crop Improvement	Vienna, Austria	Alexander Micke Joint FAO/IAEA Division P.O. Box 100 A-1400 Vienna, Austria
March 28-April 4, 1981	Federation of European Biochemical Societies	Edinburgh, United Kingdom	
April 2, 1981	Symposium on Recombinant DNA Technology	Atlanta, Georgia	American Chemical Society 1155 16th Street, N.W. Washington, D.C. 20036 (202) 872-4600
April 5-10, 1981	European Congress on Biotechnology	Eastbourne, United Kingdom	European Molecular Biology Organization
April 6-10, 1981	International Conference on Genetic Engineering	Arlington, Virginia	Melissa R. Keenberg Battelle Conference Coordination Office Suite 603 331 Madison Avenue New York, New York 10017 (212) 599-2452
April 12-17, 1981	Federation of American Societies for Experimental Biology	Atlanta, Georgia	

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
April 23-24, 1981	Recombinant DNA Advisory Committee (RAC)	Bethesda, Maryland	William J. Gartland, Director Office of Recombinant DNA Activities National Institutes of Health Bethesda, Maryland 20205 (301) 496-6051
April 24-25, 1981	Annual Meeting of the Illinois State Academy of Science: Genetic Engineering	Illinois Wesleyan University Bloomington, Illinois	L.E. Crofutt Botany Department Eastern Illinois University Charleston, Illinois 61920
May 3-16, 1981	Current Methods in Structural Moleculelar Biology NATO Advanced Study Institute	Maratea, Italy	NATO Scientific Affairs Division B1110 Brussels, Belgium
May 4-6, 1981	Genetic Engineering for Chemists and Chemical Engineers	Pasadena, California	James E. Bailey/Judith L. Campbell California Institute of Technology Pasadena, California 91125 (213) 356-4116
May 7-8, 1981	American Chemical Society Tallahassee Subsection	Tallahassee, Florida	D.F. Detar Department of Chemistry The Florida State University Tallahassee, Florida 32306

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
May 17-20, 1981	Beltsville Symposia in Agricultural Research VI. Strategies of Plant Research	Beltsville Agricultural Research Center, Beltsville, Maryland	Dr. Albert A. Piringer BARC Symp. VI Room 130 Building 003, BARC-West U.S. Department of Agriculture Beltsville, Maryland 20705 (301) 344-3338
May 13-17, 1981	Mitochondrial Genes	Cold Spring Harbor, New York	P.O. Box 100 Cold Spring Harbor, New York, 11724 (516) 367-8397
May 20-24, 1981	RNA Tumor Virus	Cold Spring Harbor, New York	P.O. Box 100 Cold Spring Harbor, New York 11724 (516) 367-8397
May 27-June 3	Organization of the Cytoplasm	Cold Spring Harbor, New York	P.O. Box 100 Cold Spring Harbor, New York 11724 (516) 367-8397
June 7-11, 1981	Tissue Culture Association	Washington, D.C.	Local Chairman: Hope E. Hopps Bureau of Biologics Food and Drug Administration Bethesda Maryland 20014 James Duff National Cancer Institute Bethesda, Maryland

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
June 8-12, 1981	Gordon Conference on Plant Molecular Biology	Proctor Academy Andover, New Hampshire	Joe L. Key Botany Department University of Georgia Athens, Georgia 30602
June 15-19, 1981	Joint Meeting of American Society of Plant Physiologists Canadian Society of Plant Physiologists	Laval University Quebec, Canada	Dr. Leonard Beevers, Secretary American Society of Plant Physiologists Botany and Microbiology Department University of Oklahoma Norman, Oklahoma 73019
June 22-26 1981	Gordon Conference on Plant Tissue Culture	Proctor Academy Andover, New Hampshire	Frederick Meins Department of Botany University of Illinois Urbana, Illinois 61801
June 21-25, 1981	Canadian Society of Microbiologists/Canadian Phytopathological Society	Ottawa, Canada	
June 22-26, 1981	Third Cleveland Symposium on Macromolecules - Recombinant DNA	Cleveland, Ohio	Alan Walton Department of Macromolecular Science Case Western Reserve University Cleveland, Ohio 44106
August 9-13, 1981	Annual Meeting of the American Phytopathological Society	New Orleans, Louisiana	

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
August 10-14, 1981	Phytochemical Society of North America	Cornell University Ithaca, New York	L. L. Creasy Department of Pomology Cornell University Ithaca, New York 14853
August 11-16, 1981	Molecular Biology of Yeast	Cold Spring Harbor, New York	P.O. Box 100 Cold Spring Harbor, New York 11724 (516) 367-8397
August 18-23, 1981	Nucleases	Cold Spring Harbor, New York	P.O. Box 100 Cold Spring Harbor, New York 11724 (516) 367-8397
August 21-28, 1981	13th International Botanical Congress	Sydney, Australia	Executive Secretary Dr. W.J. Cram University of Sydney Sydney, N.S.W. 2006 Australia
August 25-30, 1981	Phage	Cold Spring Harbor, New York	P.O. Box 100 Cold Spring Harbor, New York 11724 (516) 367-8397
September 3-5, 1981	British Society for Cell Biology	Nottingham, United Kingdom	
September 14-17, 1981	Society for General Microbiology	Edinburgh, United Kingdom	

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
November 9-13, 1981	American Society for Cell Biology	Anaheim, California	
November 29-December 24, 1981	American Society of Agronomy Crop Science Society of America Soil Science Society of America Joint Meeting	Atlanta, Georgia	Societies Business Office 677 South Segoe Road Madison, Wisconsin 53711
1981	Yeast Molecular Biology	Cold Spring Harbor, New York	P.O. Box 100 Cold Spring Harbor, New York 11724
March 7-12, 1982	American Society for Microbiology	Atlanta, Georgia	A.L. Demain Department of Nutrition and Food Science Massachusetts Institute of Technology Cambridge, Massachusetts 02139
June 6-10, 1982	Tissue Culture Association	San Diego, California	Chairman: Ocean Pellet University of California San Diego School of Medicine La Jolla, California 92093
June 13-19, 1982	American Society of Plant Physiologists	University of Illinois Champaign, Illinois	Dr. Leonard Beevers, Secretary American Society of Plant Physiologists Botany and Microbiology Department University of Oklahoma Norman, Oklahoma 73019

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
June, 1982	Gordon Conference on Plant Molecular Biology	Proctor Academy Andover, New Hampshire	Dr. Michael Rossman Department of Biological Sciences Purdue University West Lafayette, Indiana 47907
August 8-13, 1982	IAMS International Congress of Microbiology	Boston, Massachusetts	American Society for Microbiology 1913 I Street N.W. Washington, D.C. 20006
August 22-27, 1982	International Plant Nutrition Colloquium	Warwick University Coventry, England	
August 29-September 4, 1982	21st International Horticultural Congress	Hamburg, Germany	Congress Secretariat, Hamburg Messe and Congress GMBH, Congress - Organization Postfach 30 23 60 D-2000 Hamburg 36 Federal Republic of Germany
1982	Plant Molecular Biology	Location to be selected	NATO Advanced Studies Institute F.E.B.S. Advanced Course
March 6-12, 1983	American Society for Microbiology	New Orleans, Louisiana	A.L. Demain Department of Nutrition and Food Science Massachusetts Institute of Technology Cambridge, Massachusetts 02139

APPENDIX A

TABLE A-1. (Continued)

Dates	Conference	Location	Organizer
Spring, 1983	Plant Tissue Culture	University of Tennessee Knoxville, Tennessee	Dr. Karen Hughes Botany Department University of Tennessee (615) 974-2256
June, 1983	Gordon Conference on Plant Tissue Culture	Not approved for scheduling	Dr. Alexander M. Cruickshank, Director Gordon Research Conferences Pastore Chemical Laboratory University of Rhode Island Kingston, Rhode Island 02881
August 7-12, 1983	American Society of Plant Physiologists	Colorado State University Fort Collins, Colorado	Dr. Leonard Beevers, Secretary American Society of Plant Physiologists Botany and Microbiology Department University of Oklahoma Norman, Oklahoma 73019
1983 (dates to be selected)	Tissue Culture Association	Location to be selected	
August 12-17, 1984	American Society of Plant Physiologists	University of Davis Davis, California	Dr. Leonard Brevers, Secretary American Society of Plant Physiologists Botany and Microbiology Department University of Oklahoma Norman, Oklahoma 73019

APPENDIX B

A PARTIAL LIST OF PEOPLE INTERESTED, INVOLVED, OR IMPACTED BY AGRICULTURAL GENETIC ENGINEERING

Government

Richard Hill Morris Levin	Environmental Protection Agency	Washington, D.C.
Henry Miller	Food and Drug Administration	Washington, D.C.
William Gartland Maurice Levin Daryl Banks Robert Hartley Bernard Talbot	National Institutes of Health	Washington, D.C.
Seth Parker	National Institute for Occupational Safety and Health	Washington, D.C.
Oscar Zaborsky Arthur Norberg Michael Lambert	National Science Foundation	Washington, D.C.
Seth Pauker	Occupational Safety and Health Administration	Washington, D.C.
Clarence Grogan Sue Tolin David W. Krogman Gerald Still	U.S. Department of Agriculture	Washington, D.C.
Gideon Schaeffer Dean Cress	U.S. Department of Agriculture	Beltsville, Maryland
Robert Bagwill Gerald Bjorge	U.S. Patent Office	Washington, D.C.

Academic/Research Institutions

Jack Van't Hoff Benjamin Burr Daniela Sciarky	Brookhaven National Laboratory	Upton, New York
James Bonner	California Institute of Technology	Pasadena, California
Ralph Baker	Colorado State University	Fort Collins, Colorado
Randy Henke	Comparative Research Lab University of Tennessee	Oak Ridge, Tennessee
Martin Alexander Milton Zaitlin Aladar Szalay	Cornell University	Ithaca, New York
John G. Torrey Lawrence Bogorad Fred Ausubel	Harvard University	Cambridge, Massachusetts
Lee McIntosh Peter S. Carlson Ken Sink Charles Arntzen	Michigan State University	East Lansing, Michigan
Brian A. Larkins Joe Cherry Don Foard Bill Park Stanton Gelvin	Purdue University	West Lafayette, Indiana
William Thompson Virginia Walbot Ronald W. Davis Winslow Briggs	Stanford University	Palo Alto, California
Melvin Calvin Michael Freeling	University of California	Berkeley, California
C.I. Kado Raymond C. Valentine Francisco Ayala Ledyard Stebbins Francis Broadbent Emanuel Epstein Robert Shepard Bill Rains Carol Meredith	University of California	Davis, California

Stephen H. Howell	University of California	San Diego, California
Herbert Stern		
Fred Meins	University of Illinois	Urbana, Illinois
G.F. Sprague		
C.E. Green	University of Minnesota	St. Paul, Minnesota
Burle Gengenbach		
Dennis Livingston		
Tom Guilfoyle		
Jerry Jendrisak		
Irwin Rubenstein		
John Staba		
Maureen Hanson	University of Virginia	Charlottesville, Virginia
Wayne Becker	University of Wisconsin	Madison, Wisconsin
Timothy Hall		
Winston Brill		
Hugo Dooner		
John Kemp		
Ed Sheldon		
Mary-Dell Chilton	Washington University	St. Louis, Missouri
Joe Varner		
Roger Beachy		
Michael Bevan		
Michael Thomashow	University of Washington	Seattle, Washington
Arnold Bendich		
Rose Cattolico		
Eugene Nester		
Milton Gordon		
John Boynton	Duke University	Durham, North Carolina
Nicholas Gilham		
Elizabeth Harris		
Don Bourque	University of Arizona	Tucson, Arizona
Tony Cashmore	Rockefeller University	New York, New York
Nam-Hai Chua		
Annette Coleman	Brown University	Providence, Rhode Island
Mary Conde	University of Florida	Gainesville, Florida
Daryl Pring		
Rusty Mans		

Ann Depicker Howard Goodman Patricia O'Farrell	University of California	San Francisco, California
Glenn Galau Joe Key Leon Dure Richard Meagher James Rawson	University of Georgia	Athens, Georgia
Richard Hallick	University of Colorado	Boulder, Colorado
Robert Helling	University of Michigan	Ann Arbor, Michigan
Man Mohan Johri Don Miles Joseph Polacco	University of Missouri	Columbia, Missouri
Shain-Dow Kung	University of Maryland	Baltimore, Maryland
Charles Levings John Scandalios Stuart Flashman	North Carolina State University	Raleigh, North Carolina
Robert Ludwig	University of California	Santa Cruz, California
Laurens Mets	Case Western Reserve University	Cleveland, Ohio
Peter H. Morgens Dan Klessig	University of Utah	Salt Lake City, Utah
Carl Price Ellen Reardon Aurea Vasconcelos	Rutgers University	New Brunswick, New Jersey
Ruth Sager Richard Kolodner	Sidney Farber Cancer Center	Boston, Massachusetts
Jerome Schiff Martin Gibbs	Brandeis University	Waltham, Massachusetts
Elaine Tobin Robert Goldberg Sam Wildman	University of California	Los Angeles, California
Jan Sussex Rod Dale Arthur Galston	Yale University	New Haven, Connecticut
Albert Seigel William Timberlake	Wayne State University	Detroit, Michigan

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Michael Sung	Southern Illinois University	Carbondale, Illinois
Donald Weeks Abraham Marcus	Institute for Cancer Research	Philadelphia, Pennsylvania
Ronnie Roberts	Georgia Institute of Technology	Atlanta, Georgia
Herbert Taylor	Florida State University	Tallahasse, Florida
Yu-Chen Ting	Boston College	Chestnut Hill, Massachusetts
Jim Hardin	Baylor College of Medicine	Houston, Texas
Jack Pincus	SRI Institute	Palo Alto, California
George H. Kidd Ed Lipinsky Melanie Davis	Battelle Columbus Laboratories	Columbus, Ohio
Jeff Swarz	Teknekron Research, Inc.	McLean, Virginia
	Technomic Consultants	Chicago, Illinois
Sheldon Krimsky	Tufts University	Medford, Massachusetts

Industries

Paul Hung Don Smith	Abbott Industries	North Chicago, Illinois
Daniel D. Adams Lawrence Bogorad James Shepard	Advanced Genetics Science Ltd.	Greenwich, Connecticut
David Padwa Tim Hall Dale Hanson Bob Lawrence	Agrigenetics Corporation	Sante Fe, New Mexico and Madison, Wisconsin
Phillip Filner Jane Smith Eugene Fox	Atlantic Richfield Co.	Los Angeles, California
Stephen Turner Will Burton	Bethesda Research Labs	Gaithersburg, Maryland
Charles E. Wilkes Max Roha	B.F. Goodrich Company	Cleveland, Ohio
Robert Cawthorne Walter Gilbert	Biogen, S.A.	Geneva, Switzerland
Don Renn Hugo Stange Tom Bamford	FMC Corporation	Rockland, Maine and Princeton, New Jersey
	Burpee Seed Company	Warminster, Pennsylvania
D.A. Evans W.R. Sharp	Campbell Soup Company	Cinnaminson, New Jersey
Frank M. Remley	Cargill Seed Company	Minneapolis, Minnesota
William J. Theuer Lawrence Peterson	Celanese	Summit, New Jersey
Ronald Cape Peter Farley David Mark	Cetus Corporation	Berkeley, California
Homer Le Baron	Ciba Geigy	Greensboro, North Carolina
Ori Freedman	Collaborative Genetics	Waltham, Massachusetts

Howard H. Weetall	Corning Glass Works	Corning, New York
	CPC International, Inc.	Englewood, New Jersey
	Crown Zellerbach	San Fransico, California
E.G. Tonn	CSP Foods Ltd.	Saskatoon, Saskatchewan Canada
Mark D. Pickard		
Tom W. Meyers	John Deere	Moline, Illinois
C. Wiley Hinman	Diamond Shamrock	Tuscon, Arizona
Ralph Hardy Roy Chaleff Robert Giaquinta	Dupont	Wilmington, Delaware
Irving Johnson	Eli Lilly	Indianapolis, Indiana
Elazar Rabbani Shahram Rabbini Barry Weiner	Enzo Biochem	New York, New York
H.G. Corneil	Exxon Exterprises, Inc.	Florham Park, New Jersey
Robert Swanson Robert Byrnes Herb Boyer Roberto Crea Herbert Heyneker Robert Wetzel	Genentech	San Francisco, California
Ronald Brooks	General Electric	Fairfield, Connecticut
Norm Setter	General Foods Corp.	Tarrytown, New York
John Luck Asterios Gkinis	General Mills	Minneapolis, Minnesota
Leslie Glick Vincent Simmon David Jackson	Genex	Bethesda, Maryland
John Barchilon Timothy Peoples Melvin Calvin Irving Sollins Nina Federoff James Bonner Ananda Chakrabarty	Genoco R&D	New York, New York

Larry Hunt Brian Pengilly Joe Miller	Goodyear Tire and Rubber	Akron, Ohio
Martin Apple Olaf Gamborg Tom Hiatt	International Plant Research Institute	San Carlos, California
Stan Streicher	Merck, Sharp, and Dohme	Rahway, New Jersey
Ernest G. Jaworski Zachary Wochok Keith Walker	Monsanto	St. Louis, Missouri
Jerome I. Feldman	National Patent Development Corporation	New York, New York
	Occidental Petroleum Corporation	Los Angeles, California
A. Hooker John Cross Phillip Gordon Steve Molnar Tom Rice	Pfizer	Groton, Connecticut
William L. Brown Don Duvick	Pioneer Hi-Bred International, Inc.	Des Moines, Iowa
John Hunter	Proctor and Gamble	Cincinnati, Ohio
Robert Bennet Robert Luciano	Schering-Plough	Kenilworth, New Jersey
A. J. Hale Michael Doel	G. D. Searle and Co.	Des Plaines, Illinois
Harry Green	Smith, Kline, and French	Philadelphia, Pennsylvania
Jane Aldrich David York Bill Fitzgibbons V.E. Young Gordon Cross	Standard Oil (Ohio)	Cleveland, Ohio
John Howard	Stauffer Chemical Co.	Richmond, California
Robert Nutter	Upjohn	Kalamazoo, Michigan
M.M. Abo El-Nil	Weyerhauser	Centralia, Washington

Gary Templeman Allen Wolman	Frito-Lay	Dallas, Texas
C. D. McLaughlin	Chevron	Richmond, California
Kestutis Tautvydas	3M	St. Paul, Minnesota
Robert Tuttle Ted Cayle	Kraft	Glenview, Illinois
Robert Tankersley	A. H. Robins Co.	Richmond, Virginia
Zolt Harsanyi Russell Eggers	E. F. Hutton (DNA Science)	Washington, D.C.
Franklin Pass	Molecular Genetics	Edina, Minnesota
Scott King	F. Eberstadt & Co.	New York, New York

APPENDIX D

METHODOLOGY USED TO GENERATE THE SCENARIO ON THE IMPACT OF GENETIC ENGINEERING ON AGRICULTURE 1990

We generated the scenario presented in the text by a combination of expert opinion/consensus and cross-impact analysis.

We obtained the opinions of experts in plant physiology, agricultural and energy economics, economic forecasting, agronomy, and molecular biology by asking them questions about genetic engineering and its future impacts. Table 1 is a list of the experts we polled.

We granted portions of the scenario by cross-impact analysis, a technique developed in 1966 by Theodore J. Gordon and Olaf Helmer. In 1968, Gordon and Hayward reported the first experiments with the method. The basic concept of the technique is that the occurrence of an event will affect the likelihood that other events will occur. Cross-impact analysis is designed to study the effects on the probabilities of events (e.g., developments in applied genetics and its impact in specific social domains) and the interaction of those events with each other. It is used to analyze the numerous chains of impact that can occur and to determine the overall effect of these chains on the probability that each event will occur by a specific time.

The scenario takes influencing factors into account, namely, political, social, economic, and technological. To write the scenario on genetic engineering, we generated a series of descriptive terms or phrases (descriptors). An example of descriptors corresponding to each factor is presented in Table D-2. There are cognitive linkages among these descriptors. At least three types of relationships define the linkage between any two descriptors:

- Unrelated: the occurrence of one event does not affect the probability of the second.

- Enhancing: the occurrence of one event increases the probability of the second either by enabling it to occur or by provoking its occurrence.

- Inhibiting: the occurrence of the first event decreases the probability of the second either by rendering it infeasible or by blocking or preventing its occurrence.

TABLE D-1. SCIENTISTS FROM WHOM DATA AND OPINIONS FOR THE SCENARIO WERE OBTAINED

Scientist	Research Specialty	Location
Dr. Wayne Becker	Gene expression in plants, enzymology of RNA synthesis	University of Wisconsin, Madison
Melanie Davis	Plant tissue culture	Battelle Columbus Laboratories
Dr. Parviz Esmailzadeh	Operations, economic forecasting	Battelle Columbus Laboratories
Dr. Michael Evans	Plant physiology	Ohio State University
Robin Gatz	Plant genetics	Battelle Columbus Laboratories
Dr. Tim C. Hall	Molecular biology of seed proteins	University of Wisconsin, Madison
Dr. Maureen Hanson	Plant tissue culture, non-Mendelian genetics	University of Virginia
Dr. George Kidd	Plant tissue culture	Battelle Columbus Laboratories
Dr. Steve Kresovich	Agronomy of sugar crops	Battelle Columbus Laboratories
Dr. Brian Larkins	Corn seed proteins	Purdue University
Dr. Sharon Long	Symbiotic nitrogen fixation	Harvard University
Dr. Ann Matthysse	*Agrobacterium*	University of North Carolina
Dr. Don Miles	Genetics of photosynthesis	University of Missouri
Dr. Robert Miller	Soil microbiology	Ohio State University
Dr. Martin Price	Sorghum breeding	Battelle Columbus Laboratories

TABLE D-1. SCIENTISTS FROM WHOM DATA AND OPINIONS FOR THE SCENARIO WERE OBTAINED (Continued)

Scientist	Research Specialty	Location
Dr. Daryl Pring	Mitochondrial biochemistry	University of Florida
Dr. Sheldon Simon	Macroeconomics	Battelle Columbus Laboratories
Dr. Ray Valentine	Molecular biology of nitrogen fixation and salt tolerance	University of California, Davis
Alvin White	Technical economics	Battelle Columbus Laboratories

TABLE D-2. EXAMPLE DESCRIPTORS USED TO CONSTRUCT PARTS OF THE SCENARIO

Scenario Category	Descriptor
Political	Official Policies & Political Leanings U.S.-U.S.S.R.
	OECD Scientific Cooperation & Integration Warsaw-NATO Military Expenditure
Social-Economic	U.S./Western Europe Inflation/Recession Cycle
	U.S./Western Europe Policies of Free Trade
	GNP Growth in China and Japan
	GNP in U.S. and Western Europe
	Protection of Environment
	Education Policies
	Public Attitude Toward Science & Technology
Technological	Formation of Organization Engaged in Genetic Engineering
	Organic Chemical Technology
	Drug Therapy
	Conventional Breeding
	Biochemistry
	Microbiology
	Genetics of Higher Organisms

Of course, enhancing or inhibiting relationships may be of different strengths.

The linkages among descriptors can be summarized and presented in the form of a cross-impact matrix. An example in Figure D-3 shows how the linkages might be estimated for four example descriptors. In this matrix, enhancing impacts are displayed as positive numbers, with the size of the number denoting the strength of the effect. Inhibiting impacts are indicated by negative numbers and no impact by "0". A series of different scenarios can be obtained by changing the descriptors or by altering the state of the descriptors (e.g., less R&D expenditure to more R&D expenditure).

Data for the matrix are obtained from the literature and from experts in the topic area. Data from the matrix is used as an input into a computer program developed by Battelle. The program, based upon a pre- specified set of criteria, determines whether an event occurs. If it does, the probabilities of the remaining events are adjusted and the iteration is repeated until all events have been included in the analysis. Through variation of the values in the matrix and the assigned probabilities, a number of different scenarios of the possible interaction among the events is obtained.

The scenario about genetic engineering is written in a descriptive narrative style. It places the reader in the year 1990, looking back on the events of the previous ten years. It does not present the chain of events in a cause-effect manner. Rather, the relationship among the events is implied. This style has two major advantages. First, when scenarios are presented in a strict cause-effect style, the reader usually becomes entangled in one or two descriptors. Consequently, the utility of the scenario that could be derived from an appreciation of the whole, is compromised by concentrating on fine points. The descriptive narrative style provides the reader with a context before he becomes engaged with details. Second, a cause and effect argument in a scenario usually molds the imagination of the reader in advance by emphasizing a sequential line of reasoning that runs from one descriptor to another. This produces a self-imposed constraint on the part of the reader so that only the chosen descriptors presented in the scenario are emphasized as important. A descriptive scenario, however, constantly prompts the reader to enter his own variables, and to imagine alterations in the scenario.

Two assumptions were made prior to obtaining data for the cross-impact matrix.

First, genetic engineering is an incipient technology in the embryonic stages of scientific development. In the early 1980s the rate of development, direction, and magnitude of genetic engineering will be significantly influenced by political, economic, and social-cultural factors. As genetic engineering technology develops in scale and complexity and is integrated into industrial R&D efforts over the next decade, interaction among the technology and other factors becomes multi-dimensional. So, by the 1990s developments in genetic engineering technology will influence the dynamics of political, economic, and social cultural factors as much as these factors will affect the technology.

If:

The effect on this event would be:

If:	1	2	3	4
1. R&D expenditures increase	▨	+4	+1	0
2. Breakthroughs in applied genetics in industry	+4	▨	+3	+1
3. Public's rapid acceptance of products	+3	+1	▨	+3
4. Environmental dangers are ignored although dangerous accidents have occurred	0	0	-2	▨

Figure D-3. Example of a completed cross-impact matrix.

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Second, over the next ten years, developments in genetic engineering will result from a series of discontinuous and revolutionary advances and discoveries. Thus, through the 1980s the public's perception of these discoveries will determine the success of genetic engineering in the marketplace.

A scenario should not preempt a decision. It should, however, be used as a medium for stimulating a dialogue among decision makers, leading to an acceptable degree of concensus. The scenario presented in this report is as an input into policy-level decisions. The reader should be encouraged to develop his variations on the theme by altering the variables and by considering their interrelationship.

The scenario may widen the horizon of the decision makers. This is accomplished by encouraging the individual to appreciate the complexity of the future events, as well as the different ways that they might evolve. The ability to speculate ensures that decision makers will have entertained as many contingencies as possible. Scenarios facilitate this process.